

**THE USE OF RECOMBINANT HUMAN GROWTH HORMONE IN
CHILDREN WITH CHRONIC RENAL FAILURE**

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Dedication

This thesis is dedicated to my family and close friends who have provided me with encouragement and support throughout its long completion.

Declaration

I hereby declare and affirm that this thesis is entirely my own work and composition. I was the principal investigator for the three London hospitals involved in the trial. I enrolled the children into the trial and carried out all of the investigations and monitoring of this group of patients. I undertook all of the renal function tests and performed a significant proportion of the laboratory measurements myself. I co-ordinated the analyses of data from the other centres involved in the trial, and was responsible for presentation and publication of these data.

Signature

Date 20/9/00

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ABSTRACT

Poor growth is a significant problem in children with chronic renal failure. Poor nutrition and metabolic abnormalities contribute to poor growth, and in addition disturbances of growth hormone (GH) and its mediator insulin-like growth factor-I (IGF-I) have been described. Growth rates usually decline further once dialysis is required, and whilst growth rate may improve following renal transplantation in some children, this is not universal, and may not be sustained. Final adult height is reduced in children who require dialysis or transplantation during childhood.

It is on this background that recombinant human growth hormone (rhGH) is being used more commonly in children with chronic renal failure. The basis of this thesis is a multi-centre study looking at the safety and efficacy of the use of rhGH in 10 infants with chronic renal failure (CRF), 29 children with CRF, 14 children on peritoneal dialysis (PD), 8 on haemodialysis (HD) and in 22 children following renal transplantation. All of the children were followed during one year of rhGH treatment except the transplanted children who were randomised either to receive 2 years of rhGH treatment or to receive no treatment in the first year and rhGH in the second year. RhGH treatment (1iu/kg/week) improved short to medium term growth in all of these groups of children. Comparison between groups and factors predictive of the magnitude of response to rhGH are described, as are the effects of rhGH during the pubertal years.

The main safety issues which are addressed are the effects of rhGH on renal function, immune function, renal bone disease, and the effects on glucose and lipid metabolism. Patients with acromegaly, where there is overproduction of GH, have large kidneys that have an increased glomerular filtration rate (GFR). In a setting of impaired renal function, a stimulus to increase GFR could result in hyperfiltration, which is thought to be one mechanism for progression of chronic renal impairment. Renal function was measured in the CRF and transplanted children; there was no significant change in the CRF group, but an increase was seen in the transplanted group after 1 week and 6 months which returned to baseline by one year. These findings and the effects of rhGH on blood pressure, renal size and protein excretion are discussed in detail.

There are well established links between GH and the immune system, particularly in smaller mammals. In man, GH and IGF-I receptors are found on peripheral blood lymphocytes, and in vitro studies indicate a role for GH in lymphopoiesis and granulopoiesis. Minor changes in lymphocyte subsets have been reported with GH replacement in children with GH deficiency. Children with renal transplants are immunosuppressed, and any increase in immune activity could result in rejection of the graft. Flow cytometry studies demonstrated little change in lymphocyte subsets, and markers of T lymphocyte activation in the CRF and transplanted patients during rhGH treatment. Overall there was no increase in the incidence of rejection episodes in the transplanted children during rhGH treatment. These results are described in detail.

Carbohydrate and lipid metabolism are already disturbed in chronic renal failure, and could be altered further by rhGH treatment. Fasting glucose was unchanged by rhGH treatment, but fasting insulin increased transiently. Fasting cholesterol and triglyceride levels were already increased in many children before the start of treatment, and with the exception of the CRF group, in whom a small but significant increase in triglyceride was seen, there was little change during the study. The implications of these findings are discussed, as are the effects of rhGH on bone metabolism. Calcium was unchanged during treatment, but significant increases in phosphate and alkaline phosphatase were seen. Parathyroid hormone increased during rhGH treatment in the CRF groups.

Abnormalities of the GH / IGF-I axis are found in chronic renal failure; indeed a state of GH resistance has been described. Detailed studies of GH, IGF-I and its binding proteins (IGFBPs) were undertaken to investigate the mechanisms of GH resistance and the effects of rhGH treatment both before and after renal transplantation. Roles for different IGFBPs in the mechanism of short stature before and after transplantation are discussed.

Abbreviations

ALP	alkaline phosphatase
ANOVA	analysis of variance
BAPN	British Association for Paediatric Nephrology
CAPD	continuous ambulatory peritoneal dialysis
CCPD	continuous cycling peritoneal dialysis
CI	confidence interval
CRF	chronic renal failure
CRI	chronic renal insufficiency
CV	coefficient of variation
EDTA	European Dialysis and Transplant Association
ERPF	effective renal plasma flow
ESRF	end stage renal failure
FSGS	focal and segmental glomerulosclerosis
GFR	glomerular filtration rate
GH	growth hormone
GHD	growth hormone deficiency
GHRH	growth hormone releasing hormone
HbA1c	glycosylated haemoglobin
HD	haemodialysis
HLA	human leucocyte antigen
HV	height velocity
height SDS	height standard deviation score
HUS	haemolytic uraemic syndrome
HVSDS	height velocity standard deviation score
IGF-I	insulin-like growth factor-I
IGFBP	insulin-like growth factor binding protein
LRD	living related donor
mRNA	messenger ribonucleic acid

NAPRTCS	North American Pediatric Renal Transplant Co-operative Study
NK	natural killer
OGTT	oral glucose tolerance test
PAH	para-aminohippuric acid
PD	peritoneal dialysis
PTH	parathyroid hormone
rhGH	recombinant human growth hormone
RDA	recommended daily allowance
RIA	radioimmunoassay
RTA	renal tubular acidosis
SD	standard deviation
SDS	standard deviation score
SDS-PAGE	sodium dodecyl sulphate polyacrylamide gel electrophoresis
SE	standard error
WCC	white cell count
WFH	weight for height
WIB	western immunoblot
WLB	western ligand blot

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Foreword

Short stature has long been recognised as an important complication of chronic renal failure. For many children with renal disease it is the one visible sign that they are ill. Many factors, such as nutrition and electrolyte disturbances are involved, and conservative management goes some way toward improving poor growth. However many children with chronic renal failure remain short, and renal replacement therapy does not always improve the situation. Some children grow well following renal transplantation, but unfortunately this is not the case for all children. Dialysis, whether it be haemodialysis or peritoneal dialysis, may improve well-being and weight gain, but rarely results in catch up growth. More often the height drifts down through the centiles.

It is on this background that recombinant human growth hormone therapy has been introduced for children with chronic renal failure. High levels of endogenous growth hormone had initially suggested that the growth hormone / insulin-like growth factor-I axis was not involved in short stature of chronic renal failure, however in 1983 Mehls was able to demonstrate increased growth in uraemic rats given growth hormone (Mehls *et al.* 1983). These studies resulted in preliminary trials of growth hormone in children with chronic renal failure in the late 1980's and early 1990's. The beneficial results of these early studies increased the use of rhGH in short children with chronic renal failure. However, it remained necessary to investigate the safety of growth hormone treatment in these children, and to establish whether all children benefit equally from the use of growth hormone therapy.

This thesis reports the results of the British Association for Paediatric Nephrology (BAPN) multi-centre trial of recombinant human growth hormone therapy in chronic renal failure, which was co-ordinated by myself. Supplementary to the trial, further more detailed studies were undertaken to look at specific safety aspects of growth hormone treatment. I undertook these studies at the three London paediatric

nephrology centres, under the guidance of Dr L Rees, Consultant Paediatric Nephrologist.

Chapter 1: Introduction

Short stature is a significant problem for children with renal disease. Many factors contribute to the poor growth which complicates chronic renal failure (CRF), renal tubular problems, renal transplantation, and the use of corticosteroids in patients with renal disease. In certain conditions it is possible to study the isolated effect of one factor, e.g. acidosis in renal tubular acidosis, but more often there are many factors at play, and the relative importance of each is difficult, if not impossible, to assess. Age at the onset of renal problems is also an important factor, such that conditions which present in infancy are more likely to cause short stature than those which present in later life. Some renal conditions are particularly likely to be associated with growth failure. Examples of such conditions are infantile cystinosis and congenital nephrotic syndrome.

This chapter will outline normal growth during childhood, describe the patterns of growth in children with CRF, and will then go on to describe the aetiology of poor growth in renal disease, and the treatment options that are available. The rationale for the use of recombinant human growth hormone (rhGH) in this situation will be described, as will the initial results and the concerns of using rhGH in CRF. The term CRF refers to patients with a glomerular filtration rate (GFR) of less than 75 ml/min/1.73m², most of whom will be receiving conservative management only. As used this term also includes children with end stage renal failure (ESRF) (GFR of less than 10-15 ml/min/1.73m²) most of whom will have received a renal transplant or be on dialysis. In this thesis the term chronic renal insufficiency (CRI) is used to describe those children with CRF who are being treated conservatively. This term is also used to refer to the subgroup of children on conservative management who were enrolled into this trial.

Growth During Childhood

The rate of growth varies throughout childhood (Tanner *et al.* 1965), and can be divided into three stages, namely infancy, midchildhood and puberty.

Infancy

At the time of birth mean length is 50 cm and height velocity (HV) is at its greatest. Height velocity declines sharply during the first two years of life, although the rate of growth is relatively fast compared with later childhood. Approximately 26 cm of length are added during the first year of life, with a further 10 cm being gained during the second year of life. By the age of two years, a child has reached half of its final adult height, so that one third of post-natal growth has occurred in the first two years of life. Growth at this time is influenced mainly by nutrition (Betts and Magrath 1974), although there is a contribution from growth hormone (GH) (Albertsson Wikland *et al.* 1990).

Midchildhood

The rate of growth is relatively constant during midchildhood, gradually slowing down to a nadir of approximately 5 cm/yr before puberty. Growth during this time is due to the influence of GH.

Puberty

The pubertal growth spurt occurs on average after the age of 10 years in girls, and after 12 years in boys, and during puberty approximately 30 cm are added to final adult height. During the pubertal growth spurt, GH secretion increases under the influence of sex steroids.

Assessment of Growth

When assessing growth in children, it is necessary to consider both the statural height already attained and the rate of growth at the time of study. Short stature is assumed when the height is below the 3rd centile for age and sex, although by this definition

3% of normal children are included. Maintaining a position on any centile, even the 3rd centile, requires a normal height velocity; when height velocity decreases the child falls away from the centiles. To return to the normal range requires a height velocity greater than expected for age, so called catch-up growth.

Growth is expressed in cm per year (cm/yr), however as growth is seasonal, and affected by intercurrent illness or stress, it is necessary to leave a suitable interval between measurements to allow accurate estimation of annualised height velocity. This is particularly so for children who are growing poorly. Height needs to be measured at the same time of day, by trained personnel using suitably accurate equipment, such as a wall-mounted stadiometer. In the first two years of life recumbent length is measured. During this time the occipito-frontal head circumference has been shown to be a good measure of brain growth (Winick and Rosso 1969; Proyer and Thelander 1968).

For comparison of height and height velocity data, standard deviation scores (SDS) are used in preference to centiles.

$$\text{Height SDS} = \frac{\text{Patient's height} - \text{mean height for age and sex}}{\text{standard deviation for age and sex}}$$

A height SDS of zero approximates to the 50th centile and a height SDS of -2 is equivalent to a height on the 3rd centile. Height velocity SDS (HVSDS) is calculated in a similar fashion. The increase in height SDS is denoted as Δ height SDS.

Another factor which has to be taken into consideration during the assessment of growth is the bone age. Bone age reflects skeletal maturation and is an indicator of growth potential. A delayed bone age implies that the child may continue to grow for a relatively longer period of time.

Genetic factors influence height and must be considered in the assessment of a child's height. The parental heights should be measured and the mid-parental height ascertained. The final adult height for a boy should lie within 10 cm of this measurement, and for a girl within 9 cm (Tanner *et al.* 1970).

Growth in Chronic Renal Failure

Poor growth is a major problem in renal disease. Approximately 60% of children with CRI have a height below the normal range (Rizzoni *et al.* 1984; Rees *et al.* 1989). The situation is even worse for children on dialysis; the European Dialysis and Transplant Association (EDTA) registry data report a mean height SDS of -4.2 for boys and -3.8 for girls of all ages who are on dialysis (Rizzoni *et al.* 1991). Transplantation results in good catch up growth in some children (Klare *et al.* 1991; Broyer *et al.* 1992; Maxwell *et al.* 1998a), but there are many who remain below the 3rd centile. A study in Holland found that the mean final adult height SDS was -2.5 for men and women who had received renal transplants in childhood (Hokken-Koelega *et al.* 1994a), and disappointingly the North American Pediatric Renal Transplant Co-operative Study (NAPRTCS) found that catch-up growth occurred only in children aged less than 7 years at the time of transplantation (Tejani *et al.* 1993).

Short stature is a particular problem for children with congenital renal disease who are more affected than children with acquired renal disease occurring later in childhood (Betts and Magrath 1974). The type of renal disease is also important; children with inherited tubular problems are often very stunted (Potter and Greifer 1978). For many, but not all conditions, growth declines as renal function deteriorates (Betts and Magrath 1974), but with good supportive management normal growth rates can be maintained (Polito *et al.* 1987). In any one child there are multiple factors operating, and their relative importance is often difficult, if not impossible, to determine. The child's age is very important when considering the effects of renal failure on growth.

Infancy

Children with congenital renal disease and those with problems commencing in infancy are most at risk of short stature (Betts and Magrath 1974). As described above, growth is fastest in infancy; ill health at this time results in a rapid loss of stature. Antenatal diagnosis of renal problems has allowed these infants to be followed from birth; growth is most affected in the first six months of life, when height SDS can decline by as much as 0.6 SD each month (Rizzoni *et al.* 1984). The children described in this study had a normal birth weight and length. Birth weight is usually normal, but a number of children with renal dysplasia are small at birth (Rees *et al.* 1989). Data on birth length are more scarce.

Similar observations of poor growth in infancy in renal disease have been reported by a number of groups (Jones *et al.* 1982; Kleinknecht *et al.* 1983; Rizzoni *et al.* 1984; Warady *et al.* 1988; Rees *et al.* 1989; Abitol *et al.* 1993). Taking these reports together, height SDS at presentation is negatively correlated with age; mean height SDS for those presenting in the first month was -0.6 SD (Warady *et al.* 1988), while those presenting at 1 year had a mean height SDS of -2.9 (Rees *et al.* 1989).

Growth in infancy is highly dependent on nutrition (Betts and Magrath 1974), and provision of adequate calories can improve growth (Strife *et al.* 1986). Many of these infants have tubular dysfunction, and correction of electrolyte, base and fluid loss results in a marked improvement in growth (Rees *et al.* 1989). Each of these factors is discussed in detail below. In this age group, catch-up growth can be achieved in some infants with good conservative management and intensive nutritional support (Rees *et al.* 1989).

In addition to poor growth, ESRF in infancy has been described as causing an encephalopathy, manifest by reduced cognitive ability, microcephaly and reduced motor function (Rotondo *et al.* 1982; McGraw and Haka-Ikse 1985; Polinsky *et al.* 1987). Renal replacement therapy can alleviate this, with a slight improvement occurring on peritoneal dialysis (Warady *et al.* 1988), and a more marked

improvement being seen after renal transplantation (Nevins 1987; So *et al.* 1987; Davis *et al.* 1990; Najarian *et al.* 1990).

Midchildhood

After the first one to two years of life, children with CRF grow at a normal rate, so that the child remains below but parallel to the 3rd centile. Catch-up growth is rare. Rees *et al.* found that after infancy, intensive conservative management only resulted in a slow improvement in growth (Rees *et al.* 1989). At presentation to the clinic, 61% of the children described were below the normal range for height; at the end of the study period, 53% remained below the 3rd centile.

During childhood, there is no definite correlation between growth in patients with CRF and absolute GFR (Rees *et al.* 1989). Growth tends to be along the centiles at this stage (Betts and Magrath 1974; Claris-Appiana *et al.* 1989), and the growth rate remains relatively normal until either ESRF or puberty is reached. Growth on dialysis is less than that observed during conservative management (Kleinknecht *et al.* 1980). Children with a later onset of renal problems often show a slowing down of growth in the initial stages of the illness, then grow along a lower centile when their condition is more stable.

Puberty

Puberty is associated with development of secondary sexual characteristics and an increase in growth rate. The onset of the pubertal growth spurt, which is taken as the time of minimum HV prior to the growth spurt, is delayed by an average of approximately two and a half years in both sexes in renal failure (Schaefer *et al.* 1990). Interestingly the absolute height at which this occurs is similar to that in normal controls, but the peak velocity achieved during puberty is reduced to approximately 75% of that of controls. In addition the duration of the pubertal growth spurt is attenuated; on average it is reduced by one year in boys and by one and a half years in girls. These factors combine to result in a reduction in expected height gain during puberty, from 30 to 17 cm in boys and from 29 to 14 cm in girls (Schaefer *et*

al. 1990). Bone age at the end of puberty was less than expected; little growth occurred after a mean bone age of 15.6 years in boys and 14.7 in girls in this study (Schaefer *et al.* 1990). Growth potential is therefore lost during puberty.

Much of the data regarding pubertal growth and development are derived from studies which combine children on different treatment modalities. In the study described above the majority of children were receiving either conservative management or dialysis at the onset of puberty, but treatment modality changed in a number of children such that by the end of puberty most children had received a renal transplant (Schaefer *et al.* 1990). This course is not unusual in children with renal disease, so data on the effects of different treatment modalities on pubertal growth are hard to come by. Broyer described 17 children on haemodialysis, of whom 10 were of a pubertal age; the pubertal growth spurt was absent or severely depressed in all 10 children (Broyer *et al.* 1974). There is an ongoing multi-national study of growth and development during puberty in renal disease which may be able to provide these data (Scharer *et al.* 1990).

Traditionally growth during puberty in children with renal transplants has been described as poor, with some authors reporting little growth in those with a bone age over 12 years (Grushkin *et al.* 1973; Rees *et al.* 1988). Since the introduction of cyclosporin A and the subsequent reduction in steroid dosage, normal patterns of pubertal growth have been described (Maxwell *et al.* 1998a).

Sexual maturation is also delayed by approximately two and a half years in renal disease (Scharer *et al.* 1990). Progression through puberty is generally normal, although temporary maturational delay has been reported (Schaefer and Mehls 1994). Secretion of luteinising hormone is reduced in uraemic adolescents (Scharer *et al.* 1990) and adults (Rodger *et al.* 1985), but normal in transplanted patients (Scharer *et al.* 1990; Rodger *et al.* 1985). The effects of uraemia in childhood on future fertility are not known. Preliminary data suggest that sperm counts are reduced in men with renal transplants who developed ESRF in childhood (Scharer *et al.* 1989; Schaefer *et*

al. 1991a). In contrast testicular atrophy occurring in adult men who develop ESRF is reversed by successful renal transplantation (Phadke *et al.* 1970; Rodger *et al.* 1985).

Final Adult Height

The ultimate test of the management of growth is final adult height. There are only a few studies which provide this information. Changes in management over the last two decades, and an increase in the proportion of younger patients now surviving, do however limit the applicability of such data to the present time. The EDTA registry report a mean adult height SDS of -2.0 for boys and -1.3 for girls, in children who commenced renal replacement therapy before the age of 15 years and who were alive in 1984 (Rizzoni *et al.* 1986a). This report excluded patients with cystinosis and oxalosis, who are more severely stunted. Patients requiring renal replacement therapy before the age of ten were shorter than those who started between the ages of ten and fifteen. Patients who remained on dialysis were shorter than those who received renal transplants (Rizzoni *et al.* 1986a).

Final adult height SDS was less severely retarded at -0.8 in a cohort of 22 patients who remained on conservative management throughout puberty (Schaefer *et al.* 1989).

Aetiology of Poor Growth in Renal Disease

Nutrition

Currently much emphasis is attached to the role of inadequate nutrition as a cause of poor growth in children, and in particular, infants with renal disease. Uraemic rats eat less than *ad libitum* fed controls, and gain less weight than pair-fed controls, suggesting not only a reduced appetite, but also poor utilisation of energy (Chantler *et al.* 1974). Calorie supplementation improves but does not normalise growth in uraemic rats (Adelman and Holliday 1974; Holliday 1975).

In children with CRF, energy intake has variously been described as being correlated *with* (Simmons *et al.* 1971; Betts and Magrath 1974; Arnold *et al.* 1983) and *not* being correlated with growth (Broyer *et al.* 1974; Betts *et al.* 1977; Wass *et al.* 1977). Betts and Magrath were able to relate reduced calorie intake to poor growth in CRF; less than 80% of the recommended daily allowance (RDA) for age for energy intake was associated with a growth rate of less than 75% of that expected for age (Betts and Magrath 1974). Similarly Simmons *et al.* showed that in children on haemodialysis, only those with a calorie intake of greater than 70% of normal had normal growth (Simmons *et al.* 1971), but Broyer was unable to confirm this in his haemodialysis population (Broyer *et al.* 1974). Likewise calorie supplementation has been shown to improve growth in some groups of children, but not others (Simmons *et al.* 1971; Arnold *et al.* 1983). However Betts, in a prospective study did not find an improvement in growth with calorie supplements, although energy intake was only increased by 8.4% (Betts *et al.* 1977). Arnold, in a two year study, was able to correlate growth rate and energy intake during the first observational year. During the second year calorie supplementation was given, and whilst the growth rate increased towards that expected for age, catch-up growth did not occur. In the second year, there was no longer a correlation between growth and energy intake. In this study, intake increased from 73 to 103 % of RDA (Arnold *et al.* 1983). Sufficient energy intake therefore appears to be permissive for normal growth, but does not necessarily accelerate growth. In view of the evidence of poor utilisation of energy in uraemia, perhaps even higher intakes are needed.

There is evidence that growth can be increased by energy supplementation in infants with CRF (Rees *et al.* 1989; Ledermann *et al.* 1994). Rees was able to show that catch-up growth can occur in children who present to a paediatric nephrologist before the age of two (Rees *et al.* 1989). Similarly in another single centre report, enteral feeding of 26 infants and young children with congenital renal disease resulted in an improvement in length SDS from -2.9 to -2.0 after two years (Ledermann *et al.* 1994). A North American multicentre study however has given disappointing results; catch up growth occurred in some but not all infants (Abitol *et al.* 1993). The

management of small children with CRF continues to remain a challenge. Poor response to conservative management in some infants has resulted in a trial of the use of rhGH in this age group (Maxwell *et al.* 1996a).

The reasons for poor intake are anorexia, vomiting, altered taste sensation and sometimes inability to ingest adequate calories because of the large volumes of fluid ingested in some infants who are unable to conserve salt and water. If adequate nutrition cannot be achieved via the oral route, then nasogastric or gastrostomy feeding should be instituted. Recurrent episodes of infection or repeated operations which interrupt feeding programmes contribute to periods of poor growth.

Adequate calories are necessary for growth and anabolism; inadequate intake results in increased use of protein as an energy source. Inadequate protein intake as a cause of poor growth is uncommon these days, but was frequently seen in the past (Chantler 1984). Urinary and peritoneal protein losses can be high. Trials of dietary protein restriction show that growth and weight gain are not affected by restriction of protein to World Health Organisation safe levels of 0.8-1.1 g/kg/day (Wingen *et al.* 1991).

Acidosis

The importance of normal acid-base balance to growth is apparent from the study of children with renal tubular acidosis (RTA). Both proximal and distal RTA present with growth failure (Nash *et al.* 1972). Each of the 9 children with proximal RTA described by Nash presented within the first 18 months of life with failure to thrive and vomiting. Four children with distal RTA also presented with poor growth, but in addition had symptoms related to polyuria and polydipsia. In both groups there was a sharp increase in height velocity upon starting alkali therapy. Thereafter growth continued at a slower but improved rate. Normal height was achieved in all patients, with 3 approaching the 50th centile. Height velocity decreased in one patient in whom the parents temporarily stopped treatment (Nash *et al.* 1972). McSherry analysed 10 children with distal RTA: 6 had a height below the normal range; 4 were small but

within the normal range (McSherry 1978). Of the latter 4 patients, 2 were newly diagnosed neonates and two had previously been shown to be non-acidotic. In the group as a whole, height was inversely correlated with duration of acidosis. On starting alkali therapy, height velocity increased two to three fold and all children achieved a height within the normal range. Mean height was greater than that predicted from parental heights.

Whilst the evidence in RTA is impressive, direct evidence that acidosis is an important cause of poor growth in CRF is lacking. Poor growth and acidosis have been shown to be correlated in some studies (West and Smith 1956), but it is not clear whether this is a causal association. Correction of acidosis *per se* has not been demonstrated to improve growth in CRF.

The mechanism of poor growth in acidosis and improved growth with alkali therapy is not entirely clear. It may be a direct effect of acidosis, or related to increased excretion of sodium, potassium and calcium which occurs during acidosis. More recent work however suggests that the mechanism may be due to an effect on GH secretion (Caldas and Fontoura 1994). Growth hormone secretion in children with CRF and RTA was studied in the acidotic and non-acidotic state; mean and peak spontaneous and provoked GH secretion was reduced in both groups during acidosis. Levels of the main GH mediator, insulin-like growth factor-I (IGF-I), were also reduced during acidosis. Growth hormone secretion and IGF-I were no different from controls in the non-acidotic state (Caldas and Fontoura 1994).

This phenomenon has also been demonstrated in laboratory animals. Experimental acidosis resulted in reduced GH secretion (Challa *et al.* 1993a), reduced hepatic expression of GH receptor mRNA and hepatic IGF-I mRNA (Challa *et al.* 1993b) and reduced chondrocyte expression of IGF-I mRNA (Hanna *et al.* 1995). Poor growth in these acidotic animals is partially but not totally due to malnutrition, and cannot be overcome by either GH or IGF-I treatment (Ordóñez *et al.* 2000).

It has also been shown that administration of rhGH to human volunteers with experimentally induced chronic acidosis results in an increase in plasma bicarbonate from 14.1 mmol/l to 18.6 mmol/l (Sicuro *et al.* 1998). Children who have GH deficiency (GHD) are more likely to have a lower serum bicarbonate level than children with short stature due to other causes (Glaser *et al.* 1998).

Fluid and Electrolytes

Isolated tubular defects resulting in loss of electrolytes are associated with poor growth ; height velocity increases when these deficiencies are corrected. Such conditions point to the importance of water and certain electrolytes for growth. Children with Bartter's syndrome are short and replacement of sodium and potassium improves growth (Simopoulos 1979). Some of these children have a normal adult height, while others remain stunted. Similarly, in familial chloridorrhoia, a syndrome in which there is excessive loss of chloride in the stool, sodium chloride replacement results in improved growth (Roy and Arant 1981). A salt-depleted diet in rats is associated with poor growth (Wassner 1991). Approximately 50% of patients with nephrogenic diabetes insipidus are stunted. Increased fluid intake increases growth (Vest *et al.* 1963).

Salt and water loss frequently complicate obstructive uropathy. As with acidosis the contribution which correction of these deficiencies makes to improved growth in CRF is difficult to quantify.

Renal Osteodystrophy

Traditionally renal osteodystrophy has been assumed to be an important cause of growth failure in renal disease (Stickler and Bergen 1973). With florid renal bone disease complete cessation of growth can occur with destruction of the growth plate, or slipping of the epiphyses, but fortunately since the advent of activated vitamin D preparations, this is now an uncommon problem (Malluche and Faugere 1989).

An early report suggested that 1,25 dihydroxy vitamin D could increase growth velocity (Chan JCM *et al.* 1981), but this has not been substantiated by larger studies (Chesney 1985). Six out of 11 children studied by Chan showed an improvement in growth; many of these children had marked x-ray changes of renal bone disease prior to starting vitamin D therapy (Chan JCM *et al.* 1981). Treatment of uraemic rats with vitamin D increases but does not normalise growth velocity (Mehls *et al.* 1978). Improved growth seems to be limited to those with marked bone disease. However normal growth can occur in the presence of x-ray changes of renal osteodystrophy.

Anaemia

There is a theoretical role for anaemia in poor growth of renal disease; anaemia is associated with decreased appetite and recurrent infections. Conditions with marked anaemia e.g. thalassaemia are associated with poor growth (in the absence of iron overload), with recurrent transfusions improving growth. Poor oxygenation could be a rate-limiting step in cell proliferation. It has been difficult to look at the effects of anaemia in isolation, however the widespread availability of recombinant human erythropoietin has allowed the problem to be addressed from a different angle. Does correction of anaemia improve growth? Initial reports were encouraging (Seidel *et al.* 1991), but a large multi-centre trial study has shown no beneficial effect of erythropoietin on growth (Schaefer *et al.* 1991b).

Hormonal Factors

Levels of GH and IGF-I are normal or raised in CRF and it is only relatively recently that the GH/IGF-I axis has been implicated in short stature of renal disease (Samaan and Freeman 1970; Rees *et al.* 1990). Growth hormone is secreted in a nocturnal pulsatile fashion from the pituitary gland in response to episodic secretion of GH releasing hormone (GHRH) from the hypothalamus. Somatostatin from the hypothalamus acts to suppress the secretion of GH. Almost 50% of circulating GH is bound to a binding protein (growth hormone binding protein - GHBP), which may

represent a soluble form of the GH receptor (Baumann *et al.* 1989). Growth hormone acts on the liver to cause the secretion of IGF-I (Schoenle *et al.* 1982). This peptide is highly protein bound; six binding proteins have been identified in man (IGFBPs 1-6). The main carrier protein is IGFBP-3 which acts as a reservoir for IGF-I (Rosenfeld *et al.* 1994). Many of the anabolic actions of GH are mediated by IGF-I, but GH also acts directly at the growth end-plate to stimulate growth (Isaksson *et al.* 1982), and thirdly it stimulates the local production of IGF-I (Nilsson *et al.* 1990). The relative contributions of circulating and locally produced IGF-I to growth are not known.

In CRF the control of GH secretion is abnormal; stimulation of the pituitary results in oversecretion of GH (Tonshoff *et al.* 1990a). Overnight profiles of GH secretion show increased pulse amplitude and baseline GH (Schaefer *et al.* 1991c). The kidney is involved in clearing GH from the circulation, and this in part may explain the high baseline levels. GH binding protein is reduced in CRF (Maxwell *et al.* 1991; Tonshoff *et al.* 1997), suggesting a reduction in GH receptors and a state of GH resistance. Indeed it has been shown by Chan that there is reduced expression of hepatic GH receptors in uraemic rats, and these animals demonstrate reduced IGF-I mRNA expression in response to GH (Chan *et al.* 1993).

Another possible mechanism of growth failure in renal disease is a reduction in the bioactivity of IGF-I (Saenger *et al.* 1974). Circulating IGF-I is normal in CRF when measured by immunoassays, but low when measured by bioassay. IGF-I is lower in patients on dialysis than in those receiving conservative treatment only (Phillips and Kopple 1981).

Reduced IGF-I bioactivity has been postulated to be due to increased amounts of IGFBPs, in particular IGFBP-3 (Blum *et al.* 1991). It is thought that fragments of IGFBP-3, which are normally cleared by the kidney, accumulate in renal disease and bind to and therefore reduce the amount of free IGF-I. Other groups have not found IGFBP-3 to be significantly elevated in CRF, and other IGFBPs have been implicated (Baxter and Martin 1986; Hodson *et al.* 1992; Lee D-Y *et al.* 1993).

Steroids

Corticosteroids interfere with growth through a number of mechanisms. Spontaneous growth hormone secretion is reduced by steroids (Pantelakis *et al.* 1972), even in children on alternate day steroids (Rees *et al.* 1988). Steroids are thought to alter somatostatin tone (Wehrenberg *et al.* 1990). Increased GH secretion at the time of puberty is particularly susceptible to steroid suppression (Rees *et al.* 1988). IGF-I bioactivity is reduced in patients on steroids (Unterman and Phillips 1985); bioactivity falls within 6 hours of the steroid dose but returns to normal within 24 hours. Steroids may also reduce local IGF-I production; *in vitro* high levels of cortisol decrease skeletal IGF-I synthesis by reducing IGF-I transcription (McCarthy *et al.* 1990).

Steroids also interfere with collagen synthesis (Hyams *et al.* 1986). Type I procollagen is a by-product of collagen synthesis; levels are reduced in children with inflammatory bowel disease on daily steroids, but not in those on alternate day or on no steroids.

The relative importance of these mechanisms to poor growth during steroid therapy remains speculative.

Psychosocial

Emotional deprivation is a recognised cause of poor growth. Children suffering emotional deprivation have been shown to gain weight and grow when admitted to hospital (Green *et al.* 1984). The extra demands of a child with a chronic illness may be too great for a family already stressed by financial, housing or other worries, and the child may not receive the care and attention that they need. Rizzoni described an association between growth and psychological and socio-economic status (Rizzoni *et al.* 1986b). Parents may find it difficult to bond with an infant with severe congenital abnormalities, in whom survival is not assured, developing an ambivalence towards a child whom they have not expected to survive.

It is interesting that a small but significant increase in height velocity was seen in the placebo arm of a double-blind controlled study of the use of growth hormone in CRF (Hokken-Koelega *et al.* 1991).

Growth During Different Stages of Chronic Renal failure

Conservative Management

Approximately 60% of children with CRI have a height below the normal range (Rees *et al.* 1989). Renal dysplasia, with or without obstruction and reflux, is the most common cause of end stage renal disease in childhood, with growth often being affected in the first year of life. Intensive nutritional support, orally, by nasogastric tube or by gastrostomy feeding is imperative, particularly in the first year of life (Ledermann *et al.* 1994). After this age the effects of nutritional support are difficult to disentangle from other aspects of treatment. Calorie supplementation does not consistently result in improved growth (Betts *et al.* 1977), but poor growth can be demonstrated in children with diets deficient in calories (Betts and Magrath 1974; Simmons *et al.* 1971). As improved growth is not seen in every infant fed enterally, the use of rhGH is being evaluated in children of this age group (Fine *et al.* 1995; Maxwell *et al.* 1996c).

Correction of acidosis, fluid and electrolyte abnormalities and avoidance of secondary hyperparathyroidism and renal osteodystrophy by controlling serum phosphate level, and by the early use of activated vitamin D, are important aspects of management. Good conservative management allows normal growth, but catch-up growth is rare after infancy (Rees *et al.* 1989).

Dialysis

The use of peritoneal dialysis is becoming more common; of children under 15 on dialysis in the United Kingdom, approximately 68% are on peritoneal dialysis, and

32% on haemodialysis (Report of the BAPN, 1995). Home continuous ambulatory peritoneal dialysis (CAPD) or continuous cycling peritoneal dialysis (CCPD) is the treatment of choice for chronic dialysis. There are occasions however when haemodialysis is the only or preferred option. Poor social circumstances, persistent fluid overload, gastrointestinal abnormalities or previous gastrointestinal surgery may reduce the feasibility of peritoneal dialysis.

Despite correcting some of the metabolic abnormalities associated with renal failure, haemodialysis does not improve growth (Broyer *et al.* 1974). Broyer reported normal growth in 4 out of 17 children, with a variable reduction in growth rate being seen in the remainder. Catch-up growth was not seen. Kleinknecht reported a reduction in mean height SDS of 0.4SD per year in children on haemodialysis (Kleinknecht *et al.* 1980). Children who grew better could not be distinguished by residual renal function or dialysis prescription. As is the case for children managed conservatively (Arnold *et al.* 1983), energy was permissive for growth on dialysis, but did not induce catch-up growth (Simmons *et al.* 1971). Growth studies of children on dialysis tend to be limited by high rates of withdrawal due to transplantation.

Growth during the first year of peritoneal dialysis is reported as being stable, and better than that observed during haemodialysis (Fennell *et al.* 1984; Potter *et al.* 1986). However on more prolonged follow-up, growth rates during peritoneal dialysis decline (Fine and Mehls 1986).

Growth in infants on peritoneal dialysis receiving enteral feeds is variable (Strife *et al.* 1986; Warady *et al.* 1988; Ledermann *et al.* 2000). Warady reported four infants who started peritoneal dialysis before one month of age, who at the age of one year had a mean height SDS of -1.3. Neurological development was within the normal range in 3 and was mildly retarded in one child. Gross motor development was retarded in all infants. Mean occipito-frontal head circumference SDS was -1.4, with one patient being below the normal range (Warady *et al.* 1988). Kohaut reported on 9 infants commenced on peritoneal dialysis within the first 6 weeks of life (Kohaut *et al.* 1987);

mean height SDS was -0.8 at the start of CAPD and -1.7 after 1 year. Mean occipito-frontal head circumference SDS did not change during this time; -0.4 before and -0.5 after 1 year of CAPD. Four of the surviving 6 infants who are transplanted are developmentally normal, 2 are delayed. However Strife reported improved growth in 3 infants on peritoneal dialysis (Strife *et al.* 1986), and Brewer reported improved growth in 14 such infants (Brewer *et al.* 1986). Ledermann reported improved growth and increased occipito-frontal head circumference SDS in 20 infants on peritoneal dialysis followed up for 2 years (Ledermann *et al.* 2000). These are all small studies, with patients who no doubt differ in their clinical condition. The goal for these infants is renal transplantation; intensive nutritional management, and dialysis where necessary, are carried out in an attempt to maintain growth and development until transplantation is possible.

Renal Transplantation

The preferred mode of renal replacement therapy in childhood is transplantation, and many centres aim for pre-emptive transplantation. At present approximately 25% of children are transplanted without prior dialysis (Tejani *et al.* 1993). Whilst pre-emptive transplantation has not been shown to be of benefit in terms of post-transplant growth, it prevents the child from having a period in their life when growth is restricted.

Eight percent of transplants performed in the UK in 1992 in children under 15 years were from live related donors (LRD) (United Kingdom Transplant), in contrast to North America where 55% are from LRD (Tejani *et al.* 1993). Data from the North American Pediatric Renal Transplant Co-operative Study (NAPRTCS) report 5 year LRD patient survival of 97%, and of 94% for cadaver grafts respectively. Five year graft survival is 73% for LRD, and 59% for cadaver kidneys (Stablein *et al.* 1993). Comparable data from the EDTA reveal almost identical results (Broyer *et al.* 1993).

Growth following renal transplantation is variable, and appears to be influenced by several factors, principally steroid dose and schedule, graft function, and age at transplantation. Some groups report disappointing growth with catch-up being limited to children under the age of six or seven (Ingelfinger *et al.* 1981; Bosque *et al.* 1983; Tejani *et al.* 1993). Others report stable or improved growth with some achieving almost complete catch-up (van Diemen-Steenvorde *et al.* 1987; Rees *et al.* 1988; Klare *et al.* 1991; Maxwell *et al.* 1998a).

The growth suppressive effects of corticosteroids have long been recognised (Lam and Arneil 1968). The introduction of cyclosporin A has allowed the use of much lower doses of steroids, resulting in improved growth (Offner *et al.* 1987; Guest *et al.* 1991; Ettenger *et al.* 1991). Perhaps even more significantly, the use of the same total dose of prednisone in an alternate day regime compared to daily administration, results in improved growth (Broyer *et al.* 1992). In one study children who converted to alternate day steroids had a mean increase in height SDS of 0.5SD per year compared to a reduction of height SDS by -0.1 in patients who continued on daily prednisone. All children received alternate day steroids for the second year and growth rate continued to improve (Broyer *et al.* 1992). A recent Dutch study has also found a positive effect of alternate day steroids on growth in the first two years after transplantation (Hokken-Koelega *et al.* 1994b), and on final adult height (Hokken-Koelega *et al.* 1994a).

Even more impressive increases in height SDS are seen when steroids are withdrawn completely; Klare reported an increase of 0.8SD in the first year post transplant, with almost complete catch-up occurring over 4 years (Klare *et al.* 1991). Tejani also found an increase in height SDS on discontinuing steroids, but 9 of 23 patients had to restart prednisone after developing acute rejection episodes (Tejani *et al.* 1989).

Renal function is a further determinant of growth post transplantation. In a NAPRTCS report of growth during a two year study period, a reduction in creatinine of 90 $\mu\text{mol/l}$ was associated with a decrease in height SDS of -0.17 (Tejani *et al.*

1993). Pennisi found a reduction in growth rate when the GFR fell below 60 ml/min/1.73m² (Pennisi *et al.* 1977). Similarly poor catch-up growth in the first two years after transplantation has been associated with a GFR of less than 50 ml/min/1.73m² (Hokken-Koelega *et al.* 1994b).

Age at transplantation is important; the greatest catch-up growth is seen in infants, with an improvement in height SDS of 0.8 SD in the first year (Tejani *et al.* 1993). Catch-up growth is also reported in midchildhood, but the improvement lessens with increasing age. There are several reports of poor growth in older children who receive renal transplants (Ingelfinger *et al.* 1981; Bosque *et al.* 1983; Tejani *et al.* 1993), but others report normal growth at this age (van Diemen-Steenborde *et al.* 1987; Rees *et al.* 1988; Klare *et al.* 1991; Maxwell *et al.* 1998a).

Most reports agree that growth during puberty is a problem. There is a reduction in endogenous GH secretion, and peak height velocity during the pubertal growth spurt is negatively correlated with the cumulative dose of steroid (Schaefer *et al.* 1990, 1991c). Some authors suggest that improvement in growth after transplantation will occur only with a bone age of less than 12 years (Grushkin and Fine 1973; Fine and Ettenger 1988), but a pubertal growth spurt has been demonstrated in some patients of this age, albeit it of decreased amplitude (Schaefer *et al.* 1990; Najarian *et al.* 1990). With the increasing use of low dose, alternate day steroids, some adolescents can achieve a very normal pubertal growth spurt (Maxwell *et al.* 1998a).

The management of infants with end-stage disease is more controversial. Transplantation at this age has been reported to carry a higher operative and post-operative morbidity and mortality, with reduced graft and patient survival being reported by some (Fine and Ettenger 1988), but not all centres (Nevins *et al.* 1987; So *et al.* 1987). Impressive results have been obtained from centres specialising in transplantation in infants (Nevins *et al.* 1987; So *et al.* 1987). In these centres patient and graft survival are no different to those in older children and adolescents. The best results are seen with the use of living related donors. Following transplantation,

impressive catch-up growth is seen in infants (Tejani *et al.* 1993). Perhaps of greater importance is the improvement that is seen in indices of mental and motor development (Davis *et al.* 1990). There is also a marked improvement in occipito-frontal head circumference, suggesting increased brain growth (Winick and Rosso 1969).

European Dialysis and Transplant Association data report a mean height SDS, for children of *all* ages on dialysis, of -4.2 in boys and -3.8 in girls (Rizzoni *et al.* 1991). The equivalent figures for children with renal transplants were -2.7 and -2.3 respectively. There was little improvement over the 5 years of the study which was carried between 1985 and 1990. This may be due to an increase in the number of infants with congenital renal disease who have been taken onto end stage programmes during this time period. These children are often very short and they may mask any improvement in the management of growth over the last few years.

Recombinant Human Growth Hormone

In CRF poor growth may persist despite optimum conservative management, institution of dialysis, and for some children despite renal transplantation. Therefore the discovery that growth hormone improved growth in uraemic rats (Mehls *et al.* 1983), led to the first small trials of the use of rhGH treatment in short and poorly growing patients with CRF on conservative management, on dialysis, and in those with renal transplants (Lippe *et al.* 1988; Rees *et al.* 1990; Tonshoff *et al.* 1991a).

In addition to promoting growth, GH has many other actions, which can best be appreciated from the study of patients with GHD, and by studying the response of these patients to GH replacement. The main action of growth hormone is to stimulate longitudinal bone growth. Children with GHD are short; patients with gigantism are abnormally tall. Adult patients with acromegaly show overgrowth of certain bones resulting in large extremities and characteristic facies.

There are a number of mechanisms whereby GH is able to stimulate bone growth. Growth hormone appears to have a direct action on the growth plate. Evidence supporting this is the presence GH receptors on epiphyseal chondrocytes and osteoblasts (Werther *et al.* 1990). Furthermore, GH stimulates DNA synthesis, cell proliferation and the production of collagen matrix of cultured chondrocytes in vitro (Lindahl *et al.* 1986), and when injected into the growth plate of hypophysectomised rats GH produces longitudinal bone growth (Isaksson *et al.* 1982).

Growth hormone also acts via its mediator IGF-I; both systemic IGF-I and locally produced IGF-I stimulate longitudinal bone growth. Schlechter showed that part of the direct action of GH was due to locally produced IGF-I (Schlechter *et al.* 1986). Systemic IGF-I also induces growth as demonstrated by IGF-I treatment of children with GH receptor deficiency (Wilton 1992). Such treatment results in improved growth (Wilton 1993). However transgenic mice which over-express GH or GHRH grow faster than non-transgenic siblings, whilst transgenic mice over-expressing IGF-I grow at the same rate as litter mates (Ohlsson *et al.* 1998). Taken together, it would appear that both GH and IGF-I stimulate bone growth, although the mechanisms involved seem to be different. One hypothesis proposes that GH stimulates germinal chondrocytes to differentiate into proliferating chondrocytes which both produce and respond to local IGF-I in a paracrine or autocrine fashion (Isaksson *et al.* 1987).

In addition to bone growth, GH is also involved in bone remodelling. Osteoblasts express GH receptors (Nilsson *et al.* 1995) and GH stimulates proliferation and differentiation of osteoblasts (Ernst and Froesch 1988). Growth hormone increases the synthesis of type 1 collagen, alkaline phosphatase and osteocalcin in vitro (Kassem *et al.* 1994), and GH also stimulates the differentiation and activation of osteoclasts (Maor *et al.* 1989).

The importance of GH in bone remodelling is evident from the fact that patients with GHD have a reduced bone mineral content (Lu *et al.* 1992; Saggese *et al.* 1993). This is the case both for GHD occurring in childhood as shown in the above studies but is

also true for GHD acquired during adulthood (Holmes *et al.* 1994). Bone mineral content continues to be accrued until 25 - 30 years of age, however peak bone mineral mass is related to the duration and timing of the pubertal growth spurt (Finkelstein *et al.* 1992). Growth hormone replacement in GHD patients results in an increase in bone mineral density (Saggese *et al.* 1996).

The actions of GH are not limited to longitudinal bone growth. Patients with GHD have truncal obesity and abnormal body composition, with a decreased fat-free mass, increased body fat and reduced extracellular water. Adult patients with GHD have abnormal cardiac structure and function, and have cardiovascular risk factors such as hyperlipidaemia, decreased fibrinolysis and increased atherosclerosis. In GHD there is decreased psychological well-being and reduced exercise performance. Both GFR and ERPF are reduced.

There is also altered glucose metabolism. Patients with acromegaly have glucose intolerance and an increased incidence of diabetes (Sonksen *et al.* 1967). The actions of GH are balanced by those of insulin. Growth hormone reduces peripheral uptake of glucose and increases hepatic synthesis of glucose (Karlander *et al.* 1986), whereas insulin increases the peripheral uptake of glucose. These two forces act in concert to maintain a constant blood glucose level, despite intermittent carbohydrate loads. Excess GH secretion or administration of GH results in increased blood glucose levels, but there is usually an associated increase in insulin secretion which counteracts these effects (Salomon *et al.* 1989). Glucose intolerance or diabetes results when these compensatory mechanisms no longer operate. A further metabolic action of GH is to cause lipolysis. Growth hormone increases mobilisation of fat and promotes its use as a source of energy (Rabinowitz *et al.* 1965). Patients with GHD are short and fat; GH replacement increases lean body mass and reduces the proportion of body fat.

Efficacy of Recombinant Human Growth Hormone in Chronic Renal Failure

CRI - Conservative management

Barbara Lippe was the first to report the growth promoting effects of rhGH in children with CRI (Lippe *et al.* 1988). Five boys with congenital renal disease, with a mean age of 4.6 years were treated with rhGH (0.125 mg/kg thrice weekly) for 6 months. Calculated GFR was 18 (6) ml/min/1.73m², and height SDS -3.0 (1.0). Height velocity increased from 4.9 (1.4) cm/yr before treatment to 5.0 (1.0) during the 6 month treatment period. After 6 months of rhGH treatment, there were no significant changes in calculated GFR (17 (4) ml/min/1.73m²), glucose or insulin levels. There was no undue advancement in bone age.

Several other small series of children were reported subsequent to this, including an update of the same 5 patients after they had been treated for 1 year (Koch *et al.* 1989). Mean 1 year height velocity was 8.9 (1.2) cm/yr, and height SDS increased from -3.0 (0.7) to -2.4 (0.8) after 1 year. Weight gain increased significantly from 1.5 (0.5) kg per year before the study to 2.8 (0.8), and anthropometric studies showed that total skinfold thickness had decreased while mean mid-arm muscle circumference increased, with little overall change in mid-arm circumference. Four of the children had shown a good response, but in the other child, the increase in height velocity was only from 6.3 to 7.5 cm/yr with rhGH treatment despite a doubling of the rhGH dose after 6 months. Interestingly, he was the least stunted and the fastest growing of the group at the start of the trial.

The same group published data on the longer term effects of rhGH in CRI (Fine *et al.* 1991a). Six of the nine reported children were treated for 2 years, and 4 for 3 years. After 2 years of treatment, five of the six children had heights within the normal range. Height velocity remained above baseline for up to 3 years; mean height SDS in these 4 patients increased from -2.8 to -1.3 during this time.

Following these initial encouraging reports, several larger studies were performed. A Dutch placebo-controlled double-blind, cross-over study involving 20 patients reported conclusively that rhGH improved short-term growth in prepubertal children with CRF; an extra 2.9 (CI 2.3 - 3.5) cm of growth over 6 months was attributed to rhGH treatment (Hokken-Koelega *et al.* 1991). A larger, but uncontrolled multi-centre European Study (Van Es *et al.* 1991), involving 43 prepubertal children reported 2 year growth data for 17 children; height SDS increased from -3.1 at baseline to -1.9 after 2 years. Height velocity increased from 4.5 cm/yr before treatment to 9.5 cm/yr in the first year and 6.8 cm/yr in the second year. Six children reached end stage renal failure during the study, but there were no other adverse effects.

Subsequent studies, including the study reported in this thesis have concentrated on the safety of the use of rhGH in CRI, in particular the effects on renal function, on glucose and lipid metabolism, on renal bone disease and on the effects on the immune system. Factors which predict the magnitude of the response to rhGH have also been studied. The results of the BAPN study of rhGH in children with CRI are reported in Chapters 3 and 4 where they are discussed in detail, and compared with data published since the trial ended.

Dialysis

In 1990 and again in 1991 Tonshoff reported on prepubertal children on dialysis with growth failure. In the 1991 paper 10 children on dialysis are reported, and as with children with CRI, one year of rhGH treatment had a salutary effect on growth. Height velocity increased from 4.2 (0.9 - 5.4) to 7.3 (5.0 - 9.4) cm/yr. Height SDS one year before the study was -3.1, and changed little in the year before the study, but increased from -3.2 to -2.7 during rhGH treatment (Tonshoff *et al.* 1990b, 1991a).

There are fewer reported trials of the use of rhGH in children on dialysis. This is due in part to the fact that a high proportion of children are withdrawn when they receive

renal transplants and therefore spend less time on rhGH. However most reports in the literature are in agreement that the response of children on dialysis is less than that seen in children with CRI (Schaefer *et al.* 1994). This is discussed in greater detail in Chapter 5, where the results of the dialysis arm of the BAPN trial are reported.

Renal Transplantation

The first report of the use of rhGH post renal transplantation was published in the French literature by Rochiccioli *et al* in 1986. Increased height velocity was seen in a pubertal girl with a renal transplant treated with rhGH. Of course it is difficult to be certain whether the increased growth rate was due to GH or to the endogenous pubertal growth spurt. The first report in the English literature was published by van Dop *et al* in 1989. This group presented growth details of a prepubertal 11 year old boy, who 3 years after receiving a successful renal transplant had a height velocity of less than 2 cm/yr. He was receiving prednisolone 8 mg/m² on alternate days. During a year of rhGH treatment (2 mg x3/wk) he remained prepubertal and his height velocity increased to 8.7 cm/yr. Further reports followed shortly thereafter.

The following year a report by Rees *et al* described 12 children with renal transplants who received a year of rhGH treatment (30 IU/m²/week in 7 doses). Six children were prepubertal and 6 were pubertal. Height velocity increased from 2.3 (0.8 - 4.8) to 6.0 (2.8 - 10.9) and 3.1 (0.5 - 6.5) to 5.9 (4.6 - 7.0) cm/yr in these two groups respectively, but there was no significant change in height SDS (Rees *et al.* 1990).

The main concern in patients with renal transplants is whether a proposed therapeutic intervention will affect the graft, either through a change in GFR or by precipitating rejection episodes. In the study by Rees, the incidence of clinical rejection was no different in the year of treatment compared with the year before, although renal function deteriorated in 1 child in each group (Rees *et al.* 1990). One prepubertal child had 6 episodes of presumed rejection and her calculated GFR fell from 51 to 11 ml/min/1.73m² during the study. She had had 5 such episodes in the previous year.

One adolescent lost his graft after 3 months of rhGH; he was non-compliant with his medication. The children in the study by Rees were part of a large European multi-centre study (van Es *et al.* 1991). Virtually identical results were seen in this larger group; furthermore a significant increase in height SDS was seen in the prepubertal group after 2 years (-3.1 to -2.1, $p < 0.05$). Overall there was no change in GFR, but individual patients had increases in their serum creatinine (van Es *et al.* 1991).

Other studies reported similar findings; good short term improvements in growth rate, with occasional patients showing a rise in creatinine (Tonshoff *et al.* 1991a; Fine *et al.* 1991b). These studies however were confusing in their use of different rhGH regimens and inclusion of both prepubertal and pubertal patients. All used the equivalent of 30 units per m^2 per week, but this was given thrice weekly in the earlier patients and daily in patients enrolled at a later stage. Some studies included patients with cystinosis.

Two case reports prompted further concern, and urged for caution in the use of rhGH post transplantation. Tyden reported 2 children with stable graft function, both of whom developed biopsy proven acute rejection 5 and 7 months after starting rhGH. Both of the episodes were reversed by methylprednisolone. One child remained on rhGH and had a further episode of acute rejection 3 weeks later. Thereafter rhGH treatment was stopped (Tyden *et al.* 1990).

Schwartz described a 4.5 year old child who started rhGH 1.75 years after receiving a renal transplant. Creatinine was stable at 35 $\mu\text{mol/l}$, and she had suffered no rejection episodes. Four months later the creatinine increased to 80 $\mu\text{mol/l}$ and a renal biopsy showed acute rejection. Treatment with methylprednisolone was given and she was started on cyclosporin A. Growth hormone treatment was continued. Three months later the creatinine rose to 159 $\mu\text{mol/l}$; again acute rejection was seen on a biopsy. Growth hormone was stopped and after receiving a course of an anti-lymphocyte preparation (OKT3), the creatinine stabilised between 80 and 97 $\mu\text{mol/l}$ (Schwartz and Warady 1992).

Van Dop reported 8 children who were treated with rhGH. In one patient, creatinine increased from 160 to 260 $\mu\text{mol/l}$ during the first 3 months of rhGH; rhGH treatment was discontinued and the serum creatinine decreased to 190 $\mu\text{mol/l}$ (Van Dop *et al.* 1992).

Subsequent reports of acute rejection during rhGH treatment caused further concern and resulted in larger controlled studies including the BAPN trial reported in this thesis. This study was designed specifically to look at the safety aspects of the use of rhGH in CRF and following renal transplantation. The results of the transplant arm of the BAPN trial are reported in Chapter 6, along with a detailed review of the literature that has been published since the trial was completed.

Safety of Recombinant Human Growth Hormone

Renal Function

It is well recognised that patients with acromegaly have large kidneys which hyperfilter (Ikkos *et al.* 1956), and rhGH given to healthy adults results in an increase in effective renal plasma flow (ERPF) and GFR (Hirschberg *et al.* 1989). Transgenic mice secreting GH also show an increase in ERPF and GFR, and go on to develop glomerular sclerosis (Doi *et al.* 1988). Renal failure does not develop in acromegaly however, in the absence of hypertension and diabetes (Gershberg *et al.* 1957).

There are a number of mechanisms whereby rhGH could affect renal function. First, GH, through the action of IGF-I, increases GFR and ERPF in the normal kidney; micropuncture studies in the rat demonstrate that this is due to an effect on glomerular haemodynamics (Hirschberg *et al.* 1991). Second, altered renal function could be related to hypertrophy of the kidney; prolonged exposure to GH causes renal enlargement, as seen in acromegaly (Gershberg *et al.* 1957) and in transgenic mice secreting high levels of GH (Doi *et al.* 1988). Hypopituitary mice undergoing

unilateral nephrectomy have reduced hypertrophy of the remaining kidney (Astarabadi *et al.* 1953). Third, transgenic mice with prolonged exposure to GH (but not to IGF-I) develop glomerulosclerosis and renal failure (Doi *et al.* 1988). There are growth hormone receptors on mesangial cells, and receptors for IGF-I on proximal tubular and mesangial cells (Arnqvist *et al.* 1988; Aron *et al.* 1989; Rogers *et al.* 1989). Finally, rhGH may have an effect on the immune system, and in renal transplantation could precipitate acute rejection or cause progression of chronic rejection (Bozzola *et al.* 1991).

Metabolic Effects

There are other potential effects of rhGH treatment which require consideration in CRF patients. Glucose tolerance is altered in uraemia (DeFronzo *et al.* 1978) and might be further aggravated by rhGH treatment (Sonksen *et al.* 1967). There are pre-existing lipid abnormalities in renal disease (Querfeld 1993), and GH causes lipolysis (Rabinowitz *et al.* 1965). This subject is discussed further in Chapter 10.

Renal Bone Disease

As CRF progresses, there is reduced activation of vitamin D and retention of phosphate by the kidney. The resultant hypocalcaemia and hyperphosphataemia stimulate production of parathyroid hormone (PTH) which results in hyperparathyroidism and renal bone disease. This occurs early, when the GFR is still approximately 70 - 80 ml/min/1.73m² (Reichel *et al.* 1991). Management with dietary phosphate restriction, and administration of activated vitamin D supplements and phosphate binders, can prevent hyperparathyroidism.

The clinical manifestations of renal osteodystrophy are more apparent in a growing child where bone turnover is high compared with adult patients. Hyperparathyroidism results in osteitis fibrosa, and when this is extensive, slipping of the epiphyses may occur (Mehls *et al.* 1975).

It is conceivable that renal osteodystrophy may prevent or retarded the growth response to rhGH, or alternatively higher growth rates may aggravate renal bone disease.

Fluid Retention

Growth hormone treatment in GHD adult patients has been associated with sodium and water retention resulting in oedema (Salomon *et al.* 1989). Were this to happen in children with oliguric renal failure, fluid overload and hypertension would ensue. Monitoring of blood pressure and examination for signs of fluid overload is therefore required.

Immune Function

There is a potential for GH to have an effect on immune function. Growth hormone is by definition a mitogen, and it has known interactions with the immune system (Bozzola *et al.* 1991). There is therefore a potential risk of increased rate of transplant rejection, infection or of increased malignancy. Preliminary studies of rhGH in children with renal transplants have not reported an increase in the incidence of rejection episodes, however there are individual case reports to the contrary, as described above (Tyden *et al.* 1990; Schwartz and Warady 1992), and further study is needed to clarify whether rhGH can precipitate acute rejection episodes.

Chapter 2: British Association for Paediatric Nephrology Trial of Recombinant Human Growth Hormone in Chronic Renal Failure

Introduction

For many years the role of the GH/IGF-I axis in short stature of CRF was not appreciated, mainly due to the fact that children with CRF have high levels of endogenous circulating GH (Samaan *et al.* 1970). However in 1983, Otto Mehls was able to demonstrate the beneficial effect of rhGH in uraemic rats, and shortly thereafter the first preliminary trials of rhGH in renal failure were undertaken in children. The results were encouraging; one year of rhGH treatment resulted in an increase in height velocity. Further study was necessary. Would all patients respond equally to treatment? Would the increase in height velocity be sustained? Was it safe to use rhGH in patients with renal failure, in particular was it safe to use rhGH in children with renal transplants.

To answer some of these questions a multi-centre British trial of rhGH in renal failure was established in 1991 under the auspices of the BAPN. Four subgroups of children were studied: those on conservative management (CRI); children on haemodialysis (HD) or peritoneal dialysis (PD); and children with renal transplants. In addition infants with renal failure were studied as a separate group. The infants, children with CRI and the dialysis groups were entered into a prospective one year open-labelled study, the results of which are given in Chapters 3, 4 and 5 respectively. The transplanted children were enrolled into a two year randomised controlled open-labelled study, and are discussed in Chapter 6.

Multi-centre BAPN Trial of rhGH in Children with Chronic Renal Failure

The trial was designed to monitor both the efficacy and safety of the use of rhGH in CRF. The efficacy parameters studied were height velocity (HV) and change in standardised height (Δ height SDS) during treatment. Data were also collected on renal function, glucose metabolism, renal bone disease and adverse events, in

particular the occurrence of rejection episodes in the transplanted group. The above parameters were collected for all of the children entered into British trial.

In addition, all of the patients entered from the three London Paediatric Nephrology centres (Royal Free Hospital, Guy's and Great Ormond Street) had more detailed studies performed by myself. These additional studies are outlined in Chapters 7 to 9. For each of these studies, different subgroups of patients were included; summary data for each of these groups are given in the relevant chapters. Patient details for all 84 children entered into the trial are given in Table 2.1. More detailed information is included in the relevant chapters.

Eleven centres in the UK entered children into this trial (Table 2.2). Patient entry and exclusion criteria are listed below. More detailed descriptions of the four subgroups are included in the relevant paragraphs. Approval was granted from ethics committees at all centres. Written informed consent was obtained from all the parents or guardians. Verbal consent was obtained from the older children.

Inclusion Criteria

Children were considered eligible for entry to the trial if they fulfilled the following criteria:

- chronic renal failure
- height more than 2 SD below the mean for age and sex *or* a HV less than the 25th centile
- at least 2 previous height measurements at the renal clinic over the preceding 1 year (6 months for infants)
- normal thyroid function
- written informed consent.

Exclusion Criteria

Children were excluded from the trial for the following reasons:

- HV greater than the 75th centile during the preceding 6 months
- treatment with any form of growth hormone in the past
- a previous malignancy
- a severe congenital abnormality
- diabetes mellitus
- uncontrolled renal bone disease

Additional inclusion criteria for the subgroups are given below.

Infant Group

- CRF with a calculated GFR of less than $50 \text{ mls/min/1.73m}^2$ with or without dialysis
- bone age of less than 2 years
- height SDS less than -2SD or a declining height SDS and no improvement in growth despite correction of fluid and electrolyte and acid-base balance, bone disease and diet (including a trial of tube feeding when necessary)
- age less than 1 year at presentation of disease
- two previous height measurements in the last 6 months.

CRI Group

- calculated GFR of less than $50 \text{ ml/min/1.73m}^2$ for at least 1 year
- receiving conservative management at a paediatric nephrology centre

Dialysis Group

- haemodialysis (HD) or peritoneal dialysis (PD) for at least 6 months

Transplanted Group

- functioning renal transplant for more than 1 year
- minimum calculated GFR of 20 ml/min/1.73m²

Both prepubertal children and those already in the early stages of puberty were enrolled into the subgroups. Prepubertal boys were defined by a testicular volume of less than 4 ml and girls by breast development less than Tanner stage B2 (Marshall & Tanner 1969, 1970). Early puberty was defined in boys by a testicular volume between 4 and 10 ml inclusive, and in girls by stage B2 or B3.

Study Design

The study protocol for all patients is described below. More detailed explanations are provided for each of the subgroups in the relevant chapters. Patients were assessed for suitability prior to the trial. If the entry criteria were met, the child was enrolled and a patient number assigned. For the transplanted children patient numbers were held in the Pharmacy department. After stratification for pubertal status, randomisation was performed by opening sealed envelopes numbered in strict sequence. The envelopes contained details regarding treatment or no treatment.

All children were studied for at least one year; the transplanted children were studied for 2 years. After the trial was complete, children were able to remain on rhGH treatment on a named patient basis if they wished. Growth data during subsequent years of treatment are detailed in the relevant chapters.

Treatment

The dose of rhGH (Genotropin) used was 1iu/kg/week (0.14iu (0.05mg)/kg/day). This dose was given as 7 daily subcutaneous injections in the evening. The dose was adjusted according to weight at 3 monthly intervals. The growth standards of Tanner

and Whitehouse were used to calculate height SDS, and HVSDS (Tanner *et al.* 1966). Percentage ideal weight for height (WFH) was calculated 6 monthly.

Study Protocol

On day 1, all patients attended at 09.00 having fasted for at least 4 hours. The patients were examined, weight measured and blood pressure checked. Height was measured using a Harpenden stadiometer. Fasting blood samples were taken, and the families given instruction regarding administration of rhGH. Thereafter children were assessed 3 monthly, when again height, weight, and blood pressure were measured, and fasting blood samples taken. The occurrence of adverse events was noted.

On each occasion in all children, blood was sampled for estimation of a full blood count, urea, creatinine, electrolytes including calcium and phosphate, liver function tests including alkaline phosphatase (ALP), intact parathyroid hormone (PTH), glucose, insulin, cholesterol, triglycerides and glycosylated haemoglobin (HbA1c), and if appropriate cyclosporin level. These results are given and discussed in Chapter 10. Additional blood tests were performed depending on the subgroup. Insulin-like growth factors and IGFBPs were studied in the CRI and transplanted groups (Chapter 8). Lymphocyte subsets and markers of T cell activation were also measured in these groups (Chapter 9).

Accurate assessment of renal function was performed in the CRI and transplanted groups. Clearances of inulin and PAH were performed on day 1, after 1 week and after 1 year in the CRI group. Inulin clearances were performed on day 1, after 1 week and then 6 monthly for 2 years in the transplanted group. Further details are given in Chapter 7. Inulin clearance studies were not performed in the infant group.

Statistical Analysis

Results are expressed as mean (range) or mean (standard deviation) (SD). Where the distribution was skewed the median values are also given. Within and between group results were compared using the paired or unpaired Students *t*-test respectively. Comparison of change within the treatment and control groups was made by calculating and comparing the mean (standard error) (SE) change. Frequencies were compared using the chi-squared test. Analysis of multiple results within the same group over time was performed by analysis of variance (ANOVA). Correlations were performed using Pearson's correlation coefficient, and regression by both single and multiple linear regression analyses. Statistical significance was assumed with a p value of < 0.05 .

Table 2.1 Details of all patients entered into the British Association for Paediatric Nephrology trial of the use growth hormone in chronic renal failure

	INFANTS	CRI	DIALYSIS	TRANSPLANT
NUMBER	10	29	26	22
Boys / Girls	7 / 3	21 / 8	19 / 7	18 / 4
AGE	1.9	8.6	9.5	13.7
(Years)	(1.3 - 2.7)	(4.1 - 14.4)	(3.5 - 16.9)	(9.4 - 19.8)
GFR	14.5	19.5		50.4
mls/min/1.73m ²	(6 - 42)	(8.3 - 58.1)	16 PD / 10 HD	(18 - 105)
Height SDS	-3.3	-2.9	-3.1	-3.2
	(-4.6 to -2.0)	(-4.2 to -1.8)	(-5.3 to -1.0)	(-5.8 to -1.6)
<u>AETIOLOGY</u>				
Congenital Structural	8	25	11	14
Congenital Nephrotic Syndrome	1			1
Cystinosis				2
Glomerulonephritis			5	1
FSGS*		3		2
Reflux Nephropathy			4	
HUS**			1	1
Interstitial Nephritis			2	1
Vascular	1	1	3	

* focal segmental glomerulosclerosis ** haemolytic uraemic syndrome

Table 2.2 Paediatric nephrology centres involved in the BAPN trial

	INFANTS	CRI	DIALYSIS	TRANSPLANT
Belfast		1	1	
Birmingham	2	2	7	
Bristol	1	1	2	
Cardiff	4			
Glasgow	1	1		
London				
Great Ormond St		13		1
Guy's Hospital		4		10
Royal Free Hospital		3	3	6
Leeds		1	4	
Manchester		3	6	5
Newcastle	2		3	

Chapter 3: One Year Trial of Recombinant Human Growth Hormone in Infants with Chronic Renal Failure

Introduction

Poor growth is a particular problem for children with congenital renal disease (Betts and Magrath 1974). Growth in the first two years of life is greater than at any other time, such that by the age of two years children have already attained half of their final adult height (Tanner *et al.* 1966). Chronic ill health during this time can cause a reduction in height velocity, resulting in the height declining through the centile lines, and whilst later growth in the pre-school and early school years is often normal, catch-up growth rarely occurs (Rees *et al.* 1989; Ledermann *et al.* 1999). Chronic ill health during infancy can therefore result in a loss of height potential.

Optimum conservative management, including provision of adequate calories, correction of electrolyte disturbance and acid-base abnormalities and prevention of renal osteodystrophy goes some way towards improving growth; but many children remain below the normal range (Betts and Magrath 1974; Kleinknecht *et al.* 1983; Rizzoni *et al.* 1984; Polito *et al.* 1987; Claris-Appiani *et al.* 1989). Nasogastric or gastrostomy feeding to ensure adequate nutrition improves growth in some (Strife *et al.* 1986; Rees *et al.* 1989; Ledermann *et al.* 1999), but not all children (Abitol *et al.* 1993).

Growth in the first two years of life is strongly influenced by nutrition, however the influence of growth hormone at this age is now appreciated; infants who are later diagnosed as having growth hormone deficiency are shorter and lighter than normal (Albertsson Wikland *et al.* 1990). The data presented here are from a one year study of the use of rhGH in infants and young children with CRF, who were not growing despite good conservative management.

Methods

Entry criteria are detailed in Chapter 2; the following criteria were also observed:-

- CRF with a glomerular filtration rate of less than 50 mls/min/1.73m² with or without dialysis
- a bone age of less than 2 years
- height less than -2SD or a declining height SDS and no improvement in growth despite correction of fluid and electrolyte and acid-base balance, bone disease and diet (including a trial of tube feeding when necessary)
- age less than 1 year at presentation of disease
- two previous height measurements in the last 6 months.

Patients

Ten children (3 girls) were enrolled into the study. Two children received renal transplants after 12 days and 4 months respectively. Growth data are therefore presented only for the 8 children who completed at least 6 months of rhGH treatment. Patient details for all 10 children and for the 8 in whom growth data are presented are given in Table 3.1.

Mean gestational age was 37.2 (34 - 41) weeks (n = 10); three children were \leq 35 weeks gestation. Birth weight was available for 8 infants; six of the 8 infants had a birth weight within the normal range; one child was small for dates and one of the two premature infants had a weight above that expected for gestational age.

Growth data during the preceding 6 months were available for all 10 children. Mean height SDS 6 months prior to the study was -3.22 (-4.7 to -2.1) and -3.28 (-4.6 to -2) at entry to the study. Growth data relating to the 8 children completing the study are given in Table 3.2. Creatinine was measured at each time point during the trial. GFR was calculated using the Schwartz formula (Schwartz *et al.* 1976) at 6 monthly intervals. Metabolic data are reported in Chapter 10.

Results

Seven of the ten children completed the full year of treatment: three children were withdrawn after 12 days, 4 and 9.5 months respectively when they received renal transplants. Growth hormone treatment was stopped at that time. Growth data for the last of these three children have been annualised and are included in the analysis. No data are presented for the other two children. Two further children reached end-stage renal failure and were commenced on peritoneal dialysis, one after 4 months, the other after 10 months. These children remained on rhGH treatment and completed the study and their one year growth data have been included. Growth data are given in Table 3.2 and illustrated in Figure 3.1.

Height SDS increased significantly during rhGH treatment; Δ height SDS during the first 6 months of treatment at 0.7 (0.1 - 1.0) was greater than in the 6 months preceding the trial (0.08), $p = 0.007$. Height velocity did not change significantly, but there was a significant increase in HVSDS. This discrepancy is explained by the expected reduction in HV with age that is seen during infancy.

Response to rhGH in this group of patients was related to age, with the greatest increment in height SDS being seen in the youngest children (Figure 3.2). The growth response to treatment was unrelated to baseline GFR ($r = -0.141$).

Weight increased from 9.5 (7.5 - 13.8) kg to 10.6 (8.3 - 12.9) at 6 months and 11.7 (8.9 - 13.8) after 1 year ($p = 0.008$ v day1). Weight, expressed as % of the ideal weight for height, was 98% (79 - 118) at the start of the study, and 96% (79 - 127) after 1 year.

Two children started dialysis during the trial. No other serious adverse events occurred (Table 3.3).

Creatinine and GFR values are shown in Table 3.4. For the child who received a renal transplant at 9.5 months, and the the child who went onto dialysis at 10 months, the 9

month results are used. The child who started dialysis after 4 months of treatment has been excluded from the creatinine analysis, and for the GFR analysis, a value of zero has been used for the end of treatment GFR. There was no significant change in calculated GFR during the study, but there was a significant increase in creatinine.

Discussion

Renal failure in infancy is often complicated by poor growth. Height velocity is greater in infancy than at any other time during childhood, so ill health at this time results in a rapid decline in age-related height. This is the rationale behind the use of rhGH in this age group.

At entry to the trial, the mean height SDS was -3.3 at a mean age of just under two years, therefore indicating that significant growth delay had occurred over a relatively short period of time. This finding is supported by a prospective study of growth in infancy in CRF which reported that the major loss in height and weight SDS occurred within the first six months (Abitol *et al.* 1993); this loss can be as great as 0.6 SD per month (Rizzoni *et al.* 1984).

Traditionally growth in infancy has been thought to be influenced primarily by nutrition, but recently growth hormone has been recognised as a contributing factor at this age (Albertsson Wikland *et al.* 1990). The use of calorie supplements in infants with CRF, by nasogastric or gastrostomy feeding if necessary, does not consistently improve growth. (Strife *et al.* 1986; Abitol *et al.* 1993). The result of rhGH treatment in two boys aged less than two years with CRF, was reported in abstract form (Linne *et al.* 1992). Both showed a good response to treatment. A later abstract reported that after 28 months of rhGH treatment both boys had reached their target heights (Linne *et al.* 1996).

In the children reported here, mean height SDS did not change in the six months before the trial, while it increased from -3.3 to -2.2 during the year of rhGH

treatment. Each child showed an improvement, with four children entering the normal range. The increment in height SDS represents good catch-up growth, and interestingly the greatest improvement in height SDS was seen in the youngest children (Figure 3.2).

Despite the low height SDS before treatment, mean HVSDS was within the normal range. This suggests that reduction in height SDS had indeed started early. Furthermore remarkable catch up growth on rhGH was seen despite relatively normal HVSDS values although most values were within the upper half of the normal range. Although this is partly an auxiological phenomenon resulting from the high rates of growth seen in infancy, it appears to be easier to achieve catch-up growth with rhGH at a younger age compared with later childhood (Rees *et al.* 1990; Hokken-Koelega *et al.* 1991; Fine *et al.* 1994); a situation which is also reported following renal transplantation (Ingelfinger *et al.* 1981; Tejani *et al.* 1993).

The dramatic changes which occur in HV in the first two years of life complicate interpretation of growth data within this age group. Change in height SDS was used as a measure of the growth response, and HVSDS was used to allow comparison of height velocities at different ages. Ideally comparison should be with a placebo control group, but ethically this is difficult. With the small number of children involved in this study, it was decided to treat all children. Height SDS was used as the criteria for entry to the trial. Comparison of annualised HV at this age can be misleading because of the changing velocity, yet observing growth for 1 year to obtain an accurate velocity measurement will only lead to a delay in treatment.

There are limited published data of the use of rhGH in infants and young children. Fine published data from a controlled trial of rhGH in 30 children aged less than 2.5 years: 19 children received rhGH for 2 years; 11 received placebo (Fine *et al.* 1995). Height velocity in the first year was 14.1 cm/yr on rhGH and 9.3 in the placebo group. In the second year HV was 8.6 on rhGH and 6.9 in the control group. Over the two year period, Δ height SDS was 2.0 in the treatment group and -0.2 in the placebo

group. Height velocity, HVSDS and Δ height SDS during the study were significantly increased in the treatment group compared to the control group.

Growth after infancy, in the late pre-school and early school years, is often normal in CRF (Kleinknecht *et al.* 1983); it remains to be determined whether or not children treated in infancy with rhGH can maintain their position within the normal range without further rhGH treatment. A short course of rhGH treatment in infancy could possibly achieve as much as a longer course later in childhood. The children reported by Linne needed to remain on rhGH to sustain a normal height velocity (Linne *et al.* 1996), however normal growth rates following cessation of rhGH have also been reported (Warshaw *et al.* 1997). A larger series described by Fine found that 27% of children with CRI treated with rhGH maintained their height centile when treatment was stopped, while 73% had a deceleration in HV (Fine *et al.* 1996a)

There was no change in calculated GFR in the children who remained on conservative management. Two of the children were commenced on dialysis; it is difficult to know whether rhGH treatment was implicated. The group included several children with severely reduced renal function; several children were on call for renal transplants.

It has been much debated whether growth is related to GFR *per se*. It has been reported by one group that growth declines when the GFR falls below 25 ml/min/1.73m² (Betts and Magrath 1974), and by others that good growth can be achieved in some children even at a very low GFR (Kleinknecht *et al.* 1983; Rizzoni *et al.* 1984). We were unable to find a relationship between growth rate during rhGH treatment, although growth before treatment tended to be faster in those children with better renal function ($r = 0.640$, $p = 0.058$).

Conclusion

Growth hormone treatment for one year resulted in a marked improvement in height SDS in a group of infants and young children with CRI. Two children reached end

stage renal failure and required dialysis. Glomerular filtration rate in the remaining children was unaffected by rhGH treatment. There were no serious adverse events. The beneficial results seen in this group of young children suggests that early treatment with rhGH is worthwhile, however the optimum timing and duration of rhGH treatment are yet to be determined.

Table 3.1 Patient details in the infant group

	<i>n</i> = 10	<i>n</i> = 8
Age at diagnosis of CRI * (Months)	5.38 (0.07 - 25.1)	3.5 (0.07 - 15)
Age at start of trial (Months)	1.95 (15.6 - 32.4)	22.8 (15.6 - 32.4)
Birth weight (Kg)	3.11 (2.24 - 4.50)	3.19 (2.24 - 4.50)
GFR (ml/min/1.73m ²)	14.5 (6 - 42)	17 (9 - 42)
CRF / PD	9 / 1	8 / 0
<u>AETIOLOGY</u>		
Congenital Structural	8	6
Congenital Nephrotic Syndrome	1	1
Vascular	1	1

* GFR < 50 ml/min/1.73m²

Table 3.2 Growth data for the infant group in the year before and the year of rhGH treatment

	Day 1 (n = 8)	1 Year (n = 8)	
HtSDS	-3.3 (0.9)	-2.2 (1.0)	p = 0.0002
GFR (ml/min/1.73m ²)	18 (9 - 42)	17 (6 - 34)	p = 0.59
	Pre rhGH *	During rhGH	
HV (cm/yr)	8.6 (2.6)	10.4 (1.9)	p = 0.15
HVSDS	-1.3 (-1.2)	1.1 (1.1)	p = 0.006
Δheight SDS	0.08 (0.4)	1.10 (0.4)	p = 0.002

* Data relate to the 6 months before the trial; HV and HVSDS have been annualised

Table 3.3 Adverse events during recombinant human growth hormone treatment in the infant group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
3	3	Ankle pain	Resolved	Possibly related
	9	Failed renal transplant	Stopped rhGH	Possible
4	4	Renal transplant	Stopped rhGH	Possible
6	3	Painful left hip	Resolved	Unlikely
8	10	Commenced on peritoneal dialysis	Continued on rhGH	Possible
9	4	Commenced on peritoneal dialysis	Continued on rhGH	Possible
	8	Headaches and blurred vision - no cause found	Resolved	Possibly related - rhGH continued

Table 3.4 Renal function in the infant group during rhGH treatment

	Day 1	6 Months	1 Year	ANOVA
Creatinine (n=7)	213 (70 - 320)	259 (95 - 488)	276 (100 - 555)	$p = 0.02$
GFR (n=8)	17 (6 - 42)	15 (4 - 34)	15 (0 - 34)	$p = 0.39$

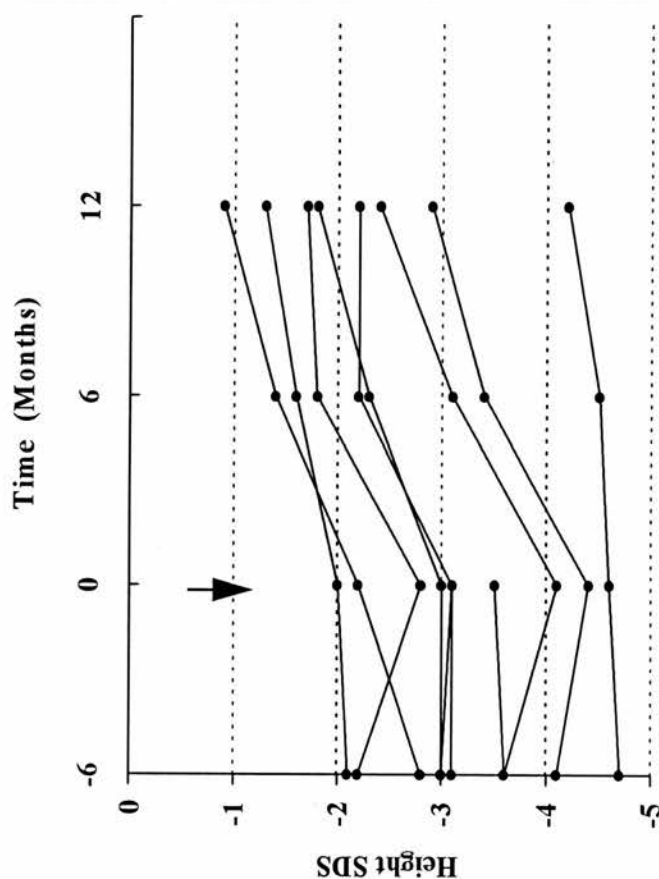


Figure 3.1 Individual height SDS measurements in the infant group 6 months before, at the start of treatment (\downarrow), and at 6 monthly intervals during rhGH treatment.

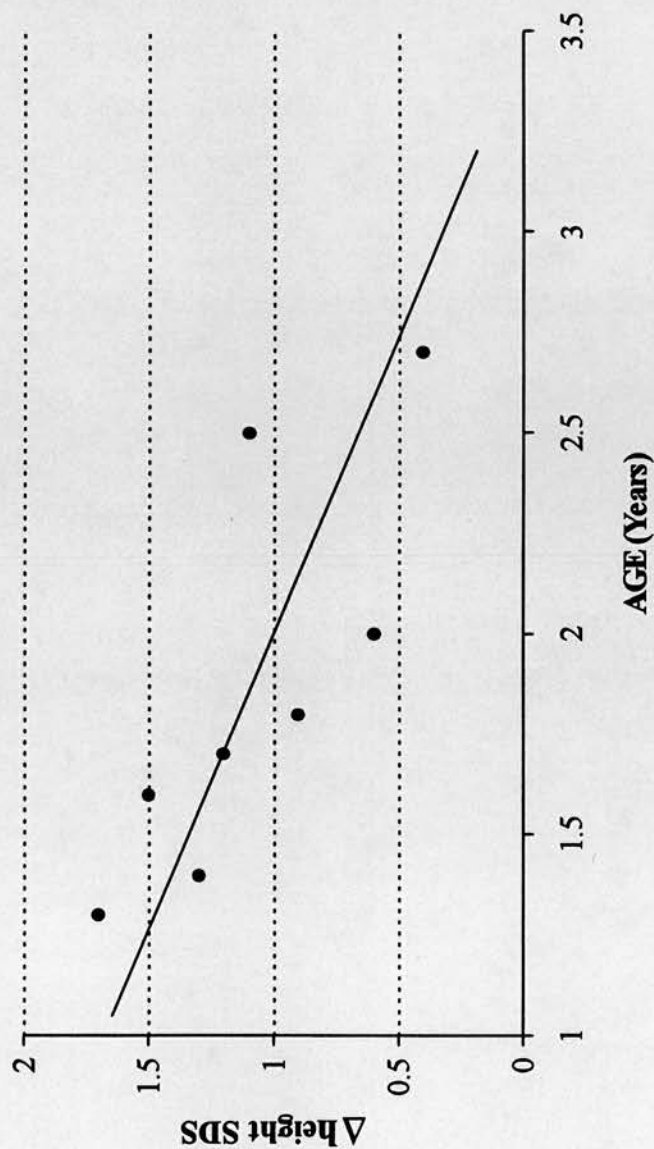


Figure 3.2 Increase in height SDS (Δ height SDS) during the year of rhGH in the infant group plotted against age at the start of treatment, $r = -0.794$, $p = 0.019$.

Chapter 4: One Year Trial of Recombinant Human Growth Hormone in Patients with Chronic Renal Insufficiency

Introduction

Almost two thirds of children with CRF managed conservatively have a height that is below the normal range (Rizzoni *et al.* 1984; Rees *et al.* 1989). As discussed in the previous chapter, growth is fastest in infancy and then slows down thereafter, such that many children with CRF grow at a normal rate, but have a height that is well below the normal range. Optimum conservative management of electrolyte disturbances, acidosis, anaemia, renal osteodystrophy and the provision of adequate nutrition improves growth in some but not all children (Claris-Appiani *et al.* 1995), however true catch-up growth is rare (Reed *et al.* 1998). Children who present to a paediatric nephrologist below the age of 2 years are more likely to show catch-up growth than those who present at a later age (Rees *et al.* 1989).

Height velocity often starts to deteriorate when the GFR falls below 50-60 ml/min/1.73m², and whilst some workers have reported that growth is proportional to the degree of renal impairment (Betts & Magrath 1974; Claris-Appiani *et al.* 1989), others have not found that to be the case (Rizzoni *et al.* 1984; Rees *et al.* 1989; Van Dyck *et al.* 1999). Even if there is no direct relationship between growth and renal function, better growth is reported in children with a GFR > 25 ml/min/1.73m² than in those with a GFR < 25 ml/min/1.73m² (Kleinknecht *et al.* 1983; Polito *et al.* 1987).

This chapter describes the effects of rhGH in children with CRI receiving conservative management. The youngest child was 4 years old. All of these children were growing poorly despite adequate medical management.

Methods

The children met the general inclusion and exclusion criteria described in Chapter 2. All had CRI with a calculated GFR of less than 50 ml/min/1.73m² (Schwartz *et al.*

1976), and required conservative management only. This was a one year study, with all participants receiving treatment with rhGH.

GFR was measured by inulin clearance on day 1 and after 1 year in 18 children from the London paediatric nephrology centres. A Schwartz formula calculated GFR was used for the remaining 9 CRI children enrolled from other centres (Schwartz *et al.* 1976).

Patients

Twenty-six prepubertal children and 3 pubertal children with CRI were entered into the trial. Patient details are given in Table 4.1. Height SDS, HV and HVSDS at the start of treatment are given in Tables 4.2 and 4.3. Eleven prepubertal children remained on rhGH after completion of the trial and growth data during this time are also reported.

Growth data are given only for those children who completed at least six months of rhGH treatment. Annualised HV during the first year of treatment was compared with the growth rate during the preceding year.

Results

Prepubertal CRI

Twenty-one of 26 prepubertal children completed 1 year of rhGH; 3 children required dialysis after 8, 9 and 10 months of rhGH, and 2 children received renal transplants after 8 and 11 months respectively. There were no other adverse events (Table 4.4). Two children entered puberty (Tanner stage II) during the study. Height velocity and HVSDS increased during treatment, and after one year there was a significant improvement in height SDS as shown in Table 4.2.

Several of the children remained on rhGH treatment after the trial was complete. Height velocity (Figure 4.1) and height SDS (Figure 4.2) remained significantly above baseline values for up to 4 years of treatment. Data from individual patients are shown in Figure 4.3. After 4 years of rhGH, 5 of 6 children had a height within the normal range, as did the 3 children who received 5 years of rhGH.

Pubertal CRI Group

All 3 pubertal children completed one year of rhGH treatment. Height velocity and height SDS increased in 2 of the 3 patients. All 3 children progressed further through puberty (Table 4.3). Individual height SDS values for these children are shown in Figure 4.4. Adverse events are shown in Table 4.5. A 15 year old pubertal girl with CRI developed weakness in both legs due to Guillain-Barre syndrome 3 months into the trial. Growth hormone treatment was continued and she made a full recovery.

Renal Function

Renal function is described in detail in Chapter 7. Creatinine was 269 (66 - 521) $\mu\text{mol/l}$ before treatment; 321 (76 - 689) after 6 months and 387 (85 - 889) after 1 year of rhGH treatment ($p < 0.0001$, ANOVA). Glomerular filtration rate was 20 (8 - 58) ml/min/1.73m^2 at the start of the study and 17 (0 - 59) after one year ($p = 0.14$).

Growth Response in All CRI Patients

Data from all 37 patients with CRI on conservative management (8 infants, 26 prepubertal and 3 pubertal patients) were combined and analysed. Age, GFR and pre-treatment parameters of growth were correlated with growth before starting treatment and with the growth response to rhGH treatment. Details are shown in Table 4.6. Before rhGH treatment there was no correlation between statural height and age, renal function nor pre-treatment HVSDS. Height velocity SDS before treatment was related to renal function, but not to age (Table 4.6).

During the first year of treatment the greatest improvement in growth rate was seen in those who were growing most slowly, Figure 4.5. The greatest increase in height SDS was seen in the youngest patients, Figure 4.6. None of the parameters of growth response to rhGH were related to GFR (Table 4.6).

Discussion

This group of children with CRI had a marked improvement in HV during the first year of rhGH treatment which resulted in a mean increase in height SDS of 0.8 SD. The greatest increase in height SDS was seen in the youngest children, although it was the children who were growing the most slowly who had the greatest improvement in growth rate.

Several children remained on rhGH after the study was complete. Height velocity remained above baseline for up to 4 years, and most of the children achieved an end of treatment height that was within the normal range. There are as yet little final adult height data for children with CRI who have been treated with rhGH, but as there is no undue advancement in bone age (Fine *et al.* 1994; Hokken-Koelega *et al.* 1994c), final height should be improved. Bone age was measured in our patients, but as these were read in different centres by different radiologists, and as the data were incomplete, height and HVSDS were calculated for chronological age.

Prior to rhGH treatment, HVSDS was faster in those with better renal function. As mentioned before, this point has previously been debated in the literature, with some authors supporting this finding (Betts and Magrath 1974; Claris-Appiani *et al.* 1989), and others refuting it (Rizzoni *et al.* 1984; Rees *et al.* 1989; Van Dyck *et al.* 1999). The children reported here are a selected group of CRI patients as they were entered into the study because of poor growth, however even within this group, we found a relationship between HVSDS and GFR.

During rhGH treatment the greatest increase in HVSDS was seen in those children who were growing most slowly prior to treatment. Similar findings were reported by the German Study Group for Growth Hormone Treatment in Chronic Renal Failure (Wuhl *et al.* 1993; Haffner *et al.* 1998a). This finding is perhaps not surprising, as the children growing most slowly may be those with the greatest degree of GH resistance, which can then be overcome with exogenous rhGH treatment. Similar findings are reported with rhGH treatment in short normal children (Hindmarsh *et al.* 1991). In this latter group, growth rate rather than height was related to GH secretion; those with the best response to rhGH were those with the lowest rates of GH secretion (Hindmarsh *et al.* 1991). Growth hormone secretion is normal in CRF, but there is evidence of GH resistance.

A Dutch study of rhGH in CRF reported a different result; they found that the increase in HVSDS was positively correlated to pre-study HVSDS (Hokken-Koelega *et al.* 1991). It is possible that this difference relates to the small number of children in the Dutch study ($n = 16$), and the fact that only data for 6 months of treatment are reported. Furthermore of the initial 20 patients, 11 were on dialysis and only 9 were receiving conservative management. It is well-recognised that the response to rhGH is less in children on dialysis than in those on conservative management. The patient group as a whole was slightly older. Recognising the potential differences between CRI and dialysis patients, we have reported the results separately.

The greatest increase in statural height (Δ height SDS) during treatment was seen in the youngest patients. This finding has also been reported in CRI (Wuhl *et al.* 1993; Haffner *et al.* 1998a), and also in children with GHD (Ranke *et al.* 1990). This may in part reflect an auxological phenomenon resulting from the faster growth rate in this group.

It is rather striking that nearly all studies of patients with CRI report a virtual doubling of HV in response to rhGH treatment; most studies report a mean HV of 9-10 cm/yr during the first year of treatment (Van Es *et al.* 1991; Tonshoff *et al.* 1992; Fine *et al.*

1994). As in this study, most authors report no relationship between growth response to treatment and renal function (Rees and Maxwell 1996a). Haffner reported a positive correlation of both Δ height SDS and Δ HVSDS during treatment and GFR (Haffner *et al.* 1998a). The difference is difficult to explain, and may partly relate to differences in patient groups; their group contained both CRI and dialysis patients.

The German Study group also reported a positive correlation with target height. This same phenomenon has been reported in GHD (Ranke *et al.* 1990). In GHD it has been suggested that the normal variation in height may relate to inherited differences in GH secretion and possibly also to an inherited variability in the response to GH. If this is indeed the case, it may also be true in CRI. In our study, parental height was recorded, but for many patients this was estimated rather than measured, and was felt to be too inaccurate for calculating target height.

Loss of height potential in renal failure occurs mainly during infancy and during the pubertal years. Two of the three pubertal children in this arm of the trial showed an increase in HV and height SDS; the other child did not. Children with CRF have a shortened and attenuated pubertal growth spurt which will reduce final adult height (Schaefer *et al.* 1990). The pubertal growth spurt in normal girls contributes 25 cm to the final height, and in boys the equivalent figure is 28cm (Tanner *et al.* 1965). If it is possible to increase pubertal height velocity in children with CRF with the use of rhGH then this may contribute to a significant increase in final adult height. However it is important to make sure that this is not achieved at the expense of a shortened pubertal growth spurt. There have been concerns that this indeed may happen in pubertal children with GHD treated with rhGH (Darendiler *et al.* 1990); there is however no data to suggest that this is the case for rhGH treatment in CRF (Hokken-Koelega *et al.* 1994c). Final adult height data in CRI patients are not yet available.

It is becoming clear that an adequate pubertal growth spurt is needed to allow accumulation of peak bone mass (Finkelstein *et al.* 1992). This is another consideration in CRI and rather than allowing these children to go on growing for

longer and have a late puberty, it may be more appropriate to induce puberty at a relatively normal time, by priming with small doses of sex hormones. A late puberty may be associated with a smaller gain in height overall (Tanaka *et al.* 1996), but a normally timed puberty may actually result in a greater increase in height gain and greater accretion of bone mass. This issue was not addressed in the present study, but needs to be a consideration in any therapeutic intervention aimed at increasing final adult height.

Height velocity was highest during the first year of treatment and as has been reported for children receiving rhGH treatment for other indications, there was a waning effect of rhGH with time (Joss *et al.* 1983; Wit 1999). Similar results in CRI have subsequently been reported by other authors (Tonshoff *et al.* 1994; Fine *et al.* 1996b). Most children show catch up growth initially and then grow parallel to the centiles. Height velocity however remained above baseline for up to 4 years, and it is possible that this will translate into an improved final height.

There are few reports of growth velocity once rhGH treatment in CRI is stopped. Fine *et al.* reported the effects of discontinuing rhGH in children who had reached their target height of mid-parental height (Fine *et al.* 1996a). Sixteen of the 22 patients reported were re-started on rhGH because of deceleration in growth rate; mean (SD) height SDS fell from -0.3 (0.8) to -0.7 (0.7) after an average of 9 (3 - 16) months. Six children remained off rhGH treatment; five of these children maintained their centile after a mean follow-up of 26 (2.4 - 58) months. Warshaw has reported one of these five children separately (Warshaw *et al.* 1997). A boy of 3 years 8 months was treated with rhGH for 21 months. Height SDS increased from -2.3 at baseline to -0.6 at the end of rhGH treatment. After nearly 5 years off treatment his height SDS was -0.7. Schaefer however reported catch-down growth in 75% of children with CRI and those on dialysis when rhGH treatment was stopped (Schaefer *et al.* 1999).

RhGH was well tolerated in this group of patients. One of the pubertal children developed Guillain Barre Syndrome after 3 months of treatment. Recovery occurred without stopping rhGH treatment, and it seems unlikely that rhGH treatment was responsible.

The other adverse events were related to renal function. Two children were on call for renal transplants and received a graft during the study. Three children had a deterioration in their GFR and were commenced on peritoneal dialysis, although overall there was no significant change in GFR. The group as a whole had poor renal function, so the withdrawals from the study were not unexpected. The effect of rhGH on GFR is addressed more fully in Chapter 7. The effects of rhGH on lipid and glucose metabolism are discussed in Chapter 10.

Conclusion

One year of rhGH treatment in a group of children with CRI managed conservatively resulted in a significant improvement in HV with no adverse effect on GFR. The greatest increase in height SDS was seen in the youngest children, although the greatest increase in HVSDS was seen in those who were growing most slowly. In children who continued on rhGH treatment, HV was increased above baseline for up to 4 years.

Table 4.1 Patient details in the CRI group

	Prepubertal	Pubertal
Number (girls)	26 (7)	3 (1)
AGE (years)	8.0 (4.1 - 13.6)	13.0, 13.9, 14.4
GFR (ml/min/1.73m²)	20 (8 - 58)	18, 14, 10
AETIOLOGY		
Congenital Structural	22	3
Focal and Segmental	3	
Glomerulosclerosis (FSGS)		
Vascular	1	

Table 4.2 Growth data for the prepubertal CRI patients in the year before and the year of rhGH treatment

	<u>Day 1</u>	<u>1 Year</u>	
Height SDS	-2.9 (0.6)	-2.1 (0.8)	<i>p</i> < 0.001
GFR (ml/min/1.73m²)	20 (8 - 58)	19 (12 - 59)	<i>ns</i>
	<u>Pre rhGH</u>	<u>During rhGH</u>	
HV	5.0 (1.5)	9.7 (2.2)	<i>p</i> < 0.001
HVSDS	-1.2 (1.4)	4.4 (2.9)	<i>p</i> < 0.001
Δheight SDS	0.0 (0.2)	0.8 (0.4)	<i>p</i> < 0.001

Table 4.3 Growth data for the pubertal CRI patients in the year before and the year of rhGH treatment.

	<u>Day 1</u>	<u>1 Year</u>
HtSDS	-2.7, -2.3, -4.2	-2.5, -1.8, -4.2
GFR	18, 14, 10	29, 11, 13
Pubertal Stage	4ml, B3, 5ml	8ml, B3, ND
	<u>Pre rhGH</u>	<u>During rhGH</u>
HV	5.0, 5.8, 3.4	7.5, 11.9, 2.0
HVSDS	-0.2, -2.0, 1.3	-0.8, 2.8, 1.1

Table 4.4 Adverse events during recombinant human growth hormone treatment in the prepubertal CRI group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
9	8.8	Commenced on peritoneal dialysis	Stopped rhGH	Possible
13	9.7	Commenced on peritoneal dialysis	Continued on rhGH	Possible
16	7.7	Renal Transplant	Stopped rhGH	None
19	11.0	Renal Transplant	Stopped rhGH	None
20	7.7	Commenced on peritoneal dialysis	Stopped rhGH	Possible

Table 4.5 Adverse events during recombinant human growth hormone treatment in the pubertal CRI group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
2	2.7	Guillain-Barre Syndrome	Resolved	Unlikely

Table 4.6 Predictors of growth before and during rhGH treatment in all CRI patients

Before rhGH Treatment

Height SDS

	r	p
AGE	0.052	0.76
GFR	0.072	0.67
HVSDS*	0.154	0.36

* during the pre-treatment year

HVSDS

	r	p
AGE	-0.191	0.26
GFR	0.308	0.016
Height SDS*	0.154	0.36

* at the start of treatment

During rhGH Treatment

ΔHVSDS

	r	p
AGE	0.227	0.18
GFR	-0.143	0.40
HVSDS*	-0.404	0.013

* during the pre-treatment year

HVSDS

	r	p
AGE	0.161	0.34
GFR	-0.014	0.93
HVSDS*	0.019	0.91

* during the pre-treatment year

Δ height SDS

	r	p
AGE	-0.577	< 0.001
GFR	-0.012	0.94
Height SDS*	-0.075	0.66

* at the start of treatment

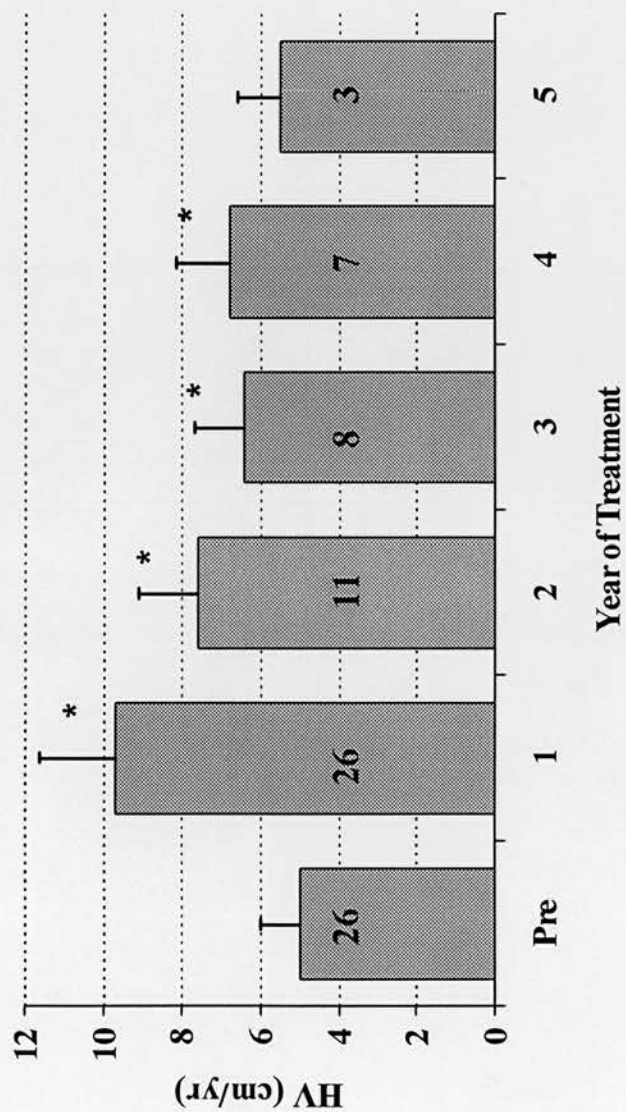


Figure 4.1 Mean (SD) height velocity (HV) in the prepubertal CRI group before and at yearly intervals after starting rhGH treatment. * $p < 0.01$ vs pre. The number of patients at each time point is shown within the bars.

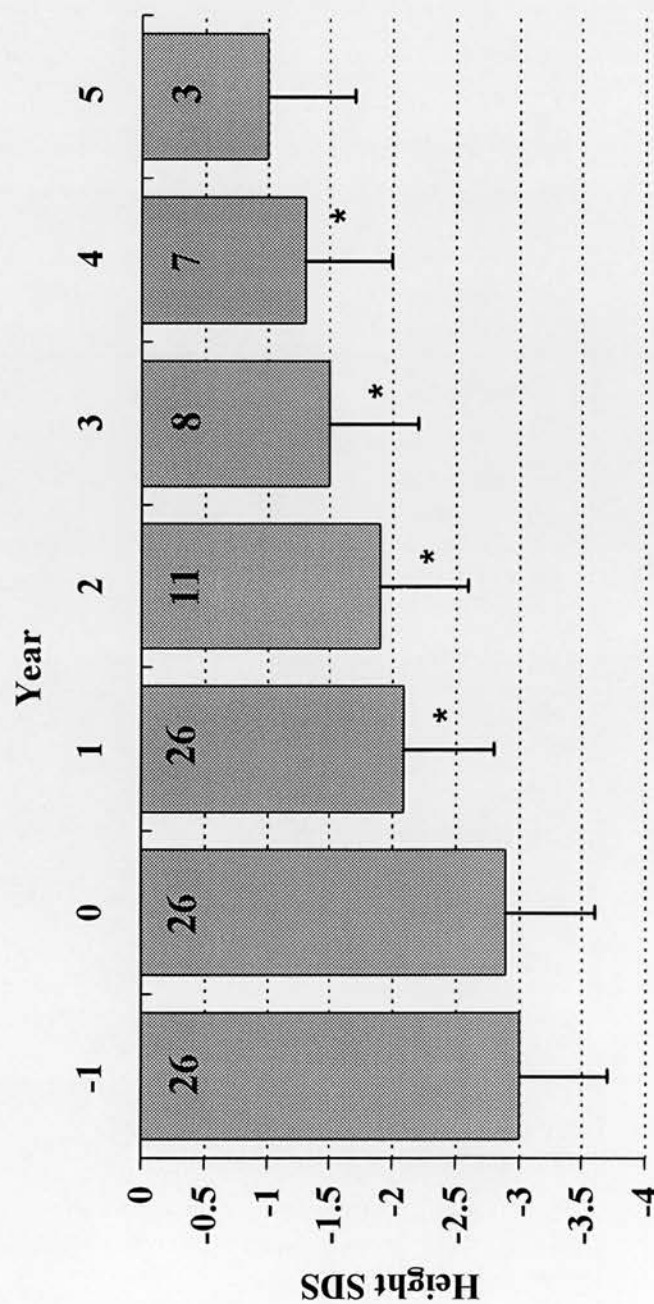


Figure 4.2 Mean (SD) height SDS in the prepubertal CRJ group one year before, at the start of treatment, and at yearly intervals thereafter. * $p < 0.01$ vs. day 1 (Time 0). The number of patients at each time point is shown within the bars.

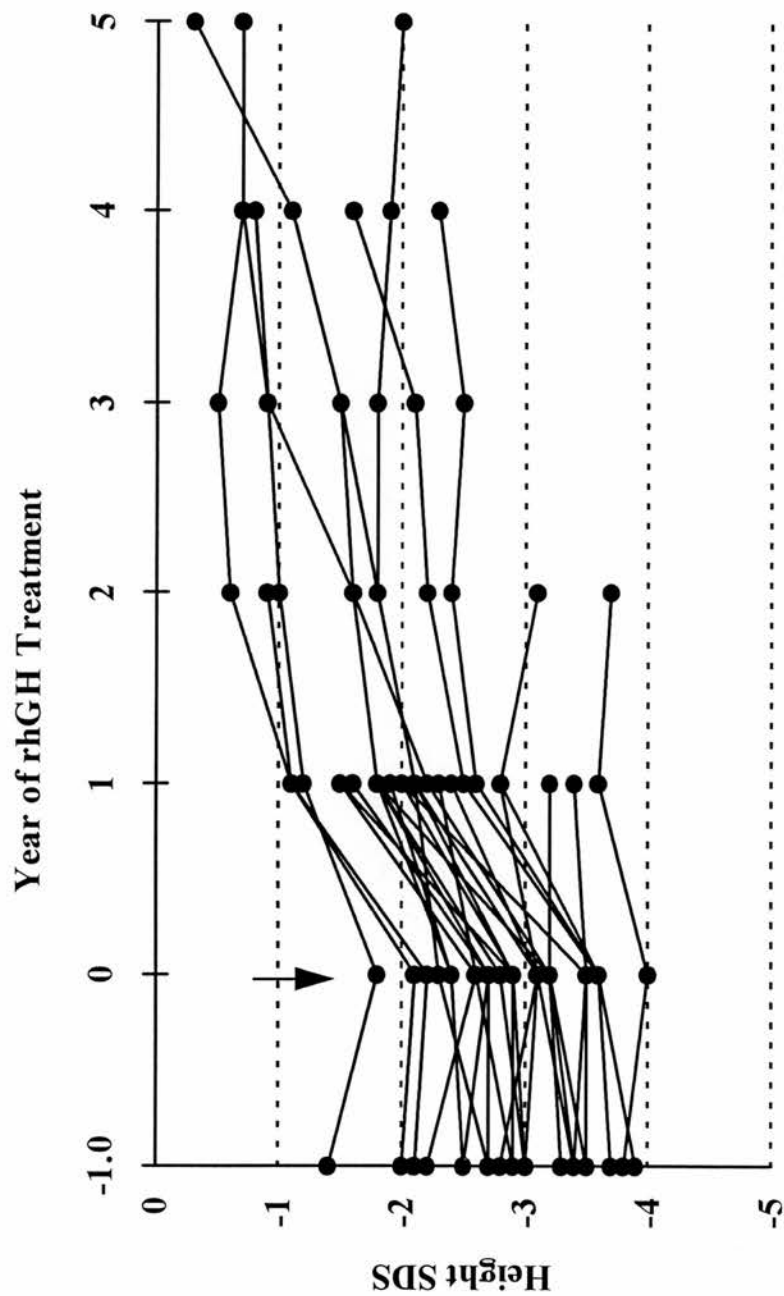


Figure 4.3 Individual height SDS measurements in the prepubertal CRI group 1 year before, at the time of starting rhGH (\downarrow), and at yearly intervals thereafter.

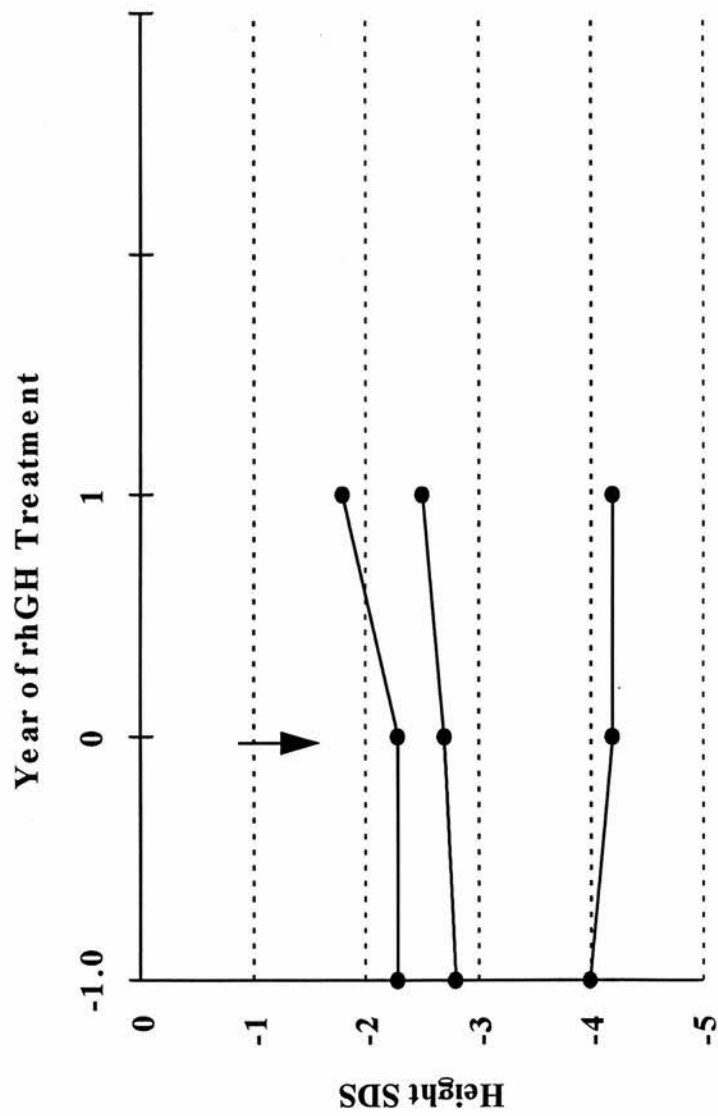


Figure 4.4 Individual height SDS measurements in the pubertal CRI group 1 year before, at the time of starting rhGH (↓), and after 1 year of treatment.

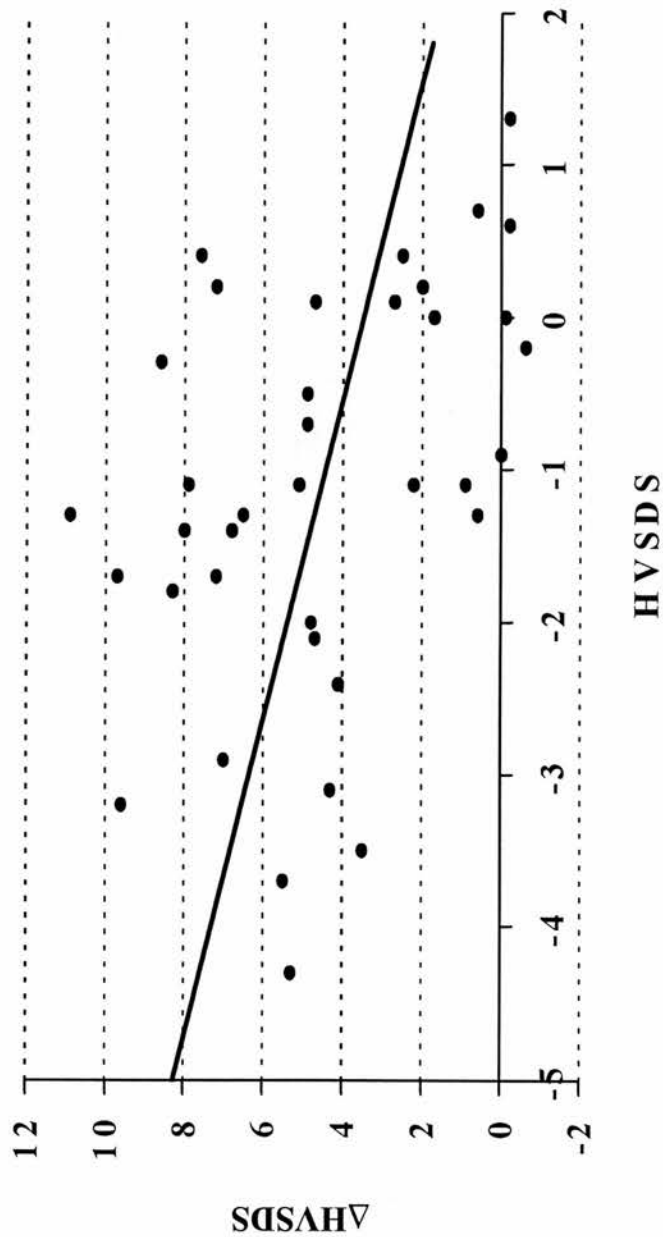


Figure 4.5 The increase in HVSDS ($\Delta HVSDS$) during the first year of rhGH treatment in all CRI children ($n=37$) plotted against HVSDS at baseline, $r = -0.404$, $p = 0.013$.

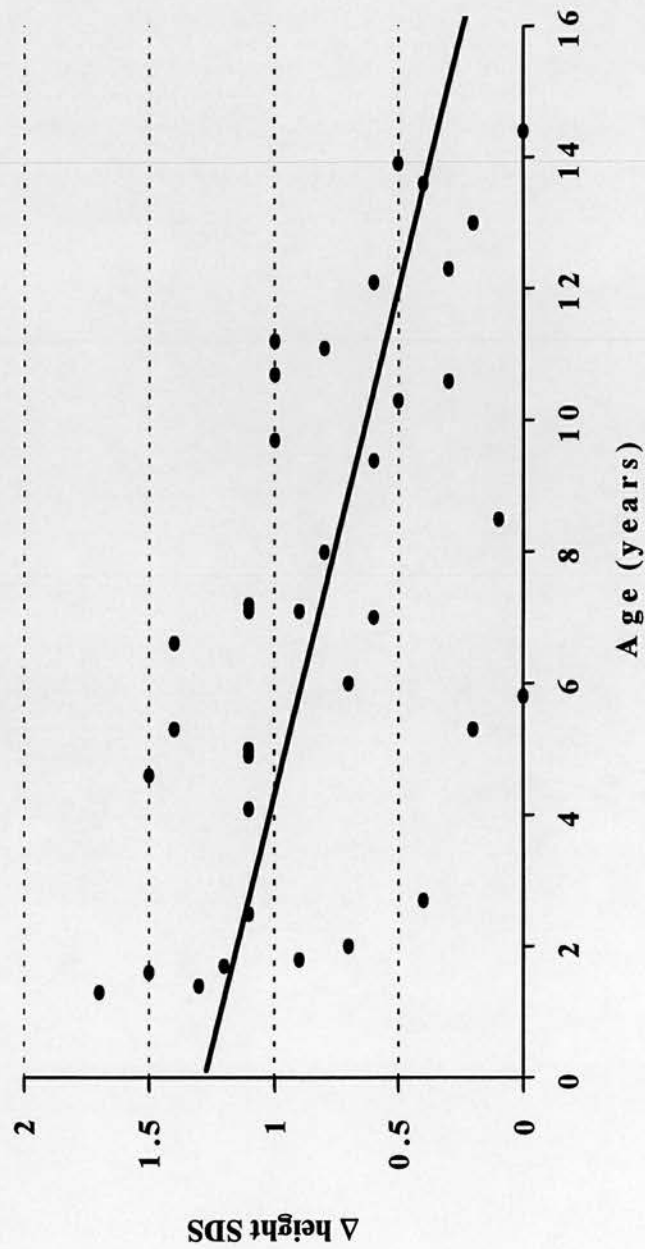


Figure 4.6 The increase in height SDS (Δ height SDS) during the first year of rhGH treatment in all CRI children ($n=37$) plotted against age at baseline, $r = -0.577$, $p < 0.001$.

Chapter 5: One Year Trial of Recombinant Human Growth Hormone In Children Undergoing Peritoneal Dialysis and Haemodialysis

Introduction

Growth in children on dialysis tends to be slower than in children with CRI or those with renal transplants. European Dialysis and Transplant Association (EDTA) registry data reported a mean height SDS of -4.2 in boys aged one to fifteen years on dialysis, and a mean height SDS of -2.7 in boys of a similar age with renal transplants (Rizzoni *et al.* 1991), and Kleinknecht reported a loss in relative height of 0.4 SD per year in children receiving long-term haemodialysis (Kleinknecht *et al.* 1980). Initial reports suggested that children on peritoneal dialysis grew better than those on haemodialysis (Fennell *et al.* 1984), but this has not been substantiated (Fine *et al.* 1986). What is clear however is that catch up growth is rare in children receiving dialysis (Broyer *et al.* 1974).

Methods

Children were eligible for entry to the trial, if in addition to the general entry criteria (Chapter 2), they had been treated with either peritoneal dialysis (n=16) or haemodialysis (n=10) for at least 6 months. All children entering the dialysis arm of the trial received one year of rhGH treatment. Increase in height SDS and HV during rhGH treatment were correlated with age and growth parameters at baseline. As some children were pubertal, HV rather than HVSDS was used.

Patients

Twenty-six children were enrolled into the dialysis study. Twenty-three were prepubertal and 3 were in early puberty (Testicular volume between 4 and 10 ml, or Breast stage 2 or 3). Patient details are shown in Table 5.1.

Results

The results are subdivided into prepubertal peritoneal dialysis, prepubertal haemodialysis and combined peritoneal and haemodialysis pubertal groups.

Prepubertal Peritoneal Dialysis

Six of the 14 children completed one year of rhGH treatment. Nine children were withdrawn; 7 received renal transplants (after 2.7, 3.1, 3.2, 3.9, 5.7, 8.0, 11.2 months respectively), one was withdrawn after developing raised intracranial pressure, and another stopped treatment 3 weeks early because of a poor response and worsening renal bone disease. These children are discussed below. The average time spent on rhGH treatment was 0.7 (0.2 - 1.2) years. All children remained prepubertal.

Growth data for the 9 children who received at least 6 months of rhGH treatment are shown in Table 5.2. Individual height SDS measurements are shown in Figure 5.1. Height SDS increased significantly in those children receiving at least 6 months of rhGH treatment; Δ height SDS was significantly greater than during the preceding year. Height velocity was on average 2.5 (0.9 - 2.5) cm greater during rhGH treatment than during the preceding year.

Eleven adverse events were reported in 7 of the 9 children in the prepubertal peritoneal dialysis group during the study. Many were almost certainly unrelated to rhGH treatment, but at least two might be causally related (Table 5.3). Patient 9, a 12 year old girl, developed headaches after 3 months of rhGH treatment. Investigation revealed raised intracranial pressure for which no cause was found. A diagnosis of benign intracranial hypertension was made. Growth hormone treatment was discontinued. She went on to make a full recovery; rhGH treatment was not re-started.

Patient 1, a 5 year old child developed worsening renal bone disease. She had a poor response to therapy. A 7 year old boy on peritoneal dialysis developed a right sided facial nerve palsy 4 days after starting rhGH followed by a left sided facial nerve palsy

one month later; both had resolved by the three month visit. The patient had also become hypertensive, which was thought to be the more likely explanation.

Prepubertal Haemodialysis Patients

Nine prepubertal children on haemodialysis were entered into the trial. Five completed the trial; four children were withdrawn when they received renal transplants (after 0, 2.2, 2.2 and 6.1 months respectively). The mean time spent on rhGH was 0.6 (0.4) years. Two children progressed into early puberty during the year of treatment.

There was no significant increase in height SDS or HV during GH therapy. Results are shown in Table 5.4. Individual height SDS measurements are shown in Figure 5.2. The change in HV during treatment compared to the previous year was 1.7 (-3.5 to 6.2) cm. The increment in height SDS was less than that seen in the prepubertal peritoneal dialysis group: 0.1 (0.3) vs. 0.4 (0.2), $p = 0.06$. Reported adverse events are shown in Table 5.5. Neither of the adverse events in this group of patients were considered to be serious.

All Prepubertal Patients

Fifteen prepubertal dialysis patients received at least 6 months of rhGH treatment. Growth data for this combined group are given in Table 5.6. Height velocity and height SDS increased significantly, although Δ height SDS was small 0.3 (-0.2 to 0.3).

Pubertal Dialysis Patients

Height velocity increased in all 3 pubertal patients; height SDS increased in two. All progressed further into puberty. Individual height SDS values for these children are shown in Figure 5.3. The mean time on rhGH was 1.0 (0.9 - 1.1) years. Growth data are shown in Table 5.7. Adverse events and their possible relationship to treatment are given in Table 5.8. None of these events were considered to be serious.

Correlations

Combining all of the dialysis patients, HV during rhGH treatment was positively correlated with pre-treatment HV, ($r=0.514$, $p=0.03$), however the greatest increase in HV during treatment was seen in those children who were growing most slowly before treatment ($r= -0.568$, $p=0.013$), Figure 5.4. Increase in height SDS during rhGH was not correlated with age ($r=0.153$) nor with pre-treatment height SDS ($r=0.034$).

Discussion

One year of treatment with rhGH improved short term growth in prepubertal children on dialysis such that height velocity and Δ height SDS increased significantly. Comparing the peritoneal dialysis and haemodialysis groups, the children on peritoneal dialysis had a better response than those on haemodialysis.

It is well recognised that children who are on dialysis grow more slowly than children with less advanced renal failure (Rizzoni *et al.* 1986b). There are a number of possible explanations for this finding. Children with ESRF are more likely to be anaemic, to have renal osteodystrophy, to be acidotic or to be less well-nourished. There is more disruption of the GH / IGF-I axis in dialysis patients compared with those with less advanced renal failure (Blum *et al.* 1991). Furthermore, children on dialysis tend to be older than those with CRI, and many have delayed puberty. Growth rate decreases with age, and growth is at its slowest in the immediate pre-puberty period, which is a time of natural resistance to the actions of endogenous GH. The older age range may partly explain why as a group children on dialysis tend to grow more slowly.

Poor growth in children on dialysis has led to the use of rhGH in an effort to improve growth. The German study group for growth hormone treatment in chronic renal failure reported the results of a 1 year study involving 14 patients on haemodialysis and 17 on peritoneal dialysis (Schaefer *et al.* 1994). These patients were treated with the same dose of rhGH as used in this study. Height velocity increased in the

haemodialysis group from 2.9 (1.9) to 5.5 (1.7) and from 3.0 (2.2) to 7.2 (3.2) cm/yr in the peritoneal dialysis group. The increase in growth rate in the peritoneal dialysis group was marginally better than in the haemodialysis group ($p=0.09$). The increments in height SDS during the first year of rhGH were 0.4 and 0.5 respectively. These results are similar to our results.

Berard reported the results of three French trials of rhGH in poorly growing children on haemodialysis (Berard *et al.* 1998). Combining the results for the first year of rhGH treatment, HV increased from 3.5 (2.2) to 7.0 (2.4) cm/yr; the corresponding increase in height SDS was 0.5 (0.5) SD. In the children who remained on rhGH treatment, HV remained above baseline values for up to 4 years of treatment (Berard *et al.* 1998). In the BAPN study it was not possible to perform such an analysis as insufficient numbers of children remained on rhGH after the trial. Most received renal transplants during or shortly after the study period.

During the first year of rhGH treatment, the fastest growth rate was seen in the children who were growing fastest at the start of the study, but the greatest increase in HV was seen in the children who were growing most slowly in the year prior to treatment. This finding has also been reported by other authors (Berard *et al.* 1998; Wuhl *et al.* 1993). Indeed Berard suggests that the value of treating patients growing faster than six cm per year is debatable.

In the French study, HV was unrelated to pubertal status (Berard *et al.* 1998); the number of patients in our study was insufficient to determine if pubertal status affected the response to treatment. Each of the 3 pubertal children showed an increase in height velocity with treatment. This is encouraging, as growth during puberty in these children can often be very poor, with a delayed and attenuated growth spurt. In the absence of a control group, it is impossible to differentiate the effects of rhGH from those of the endogenous pubertal growth spurt.

The mean increase in height SDS and HV during the first year of treatment was less than that seen in the CRI group. Other studies have reported that the response to rhGH treatment in dialysis patients is less than in conservatively managed patients. Children on dialysis tend to be shorter and to have slower growth velocities than children with conservatively managed CRI, and so it is disappointing that rhGH appears to be of less benefit in this group. There are several reasons why children on dialysis might show a poorer response to rhGH than those being managed conservatively. Children with ESRF tend to receive rhGH for shorter periods of time, and may be more likely to have intercurrent illnesses or other medical complications than children with CRI. Age has been found to be a negative predictive factor in the response to rhGH in renal failure in some studies (Wuhl *et al.* 1993), and children on dialysis tend to be older than those receiving conservative treatment. However one study compared children aged less than 10 years on dialysis with an age and sex matched group with CRI, and reported that even if one controls for age, sex, height and height velocity, children on dialysis still have a poorer response to rhGH than children with CRI (Wuhl *et al.* 1996).

A significant adverse effect occurred in one of the prepubertal peritoneal dialysis patients who developed benign intracranial hypertension. Benign intracranial hypertension has been reported previously with rhGH treatment (Malozowski *et al.* 1993, 1995), and it seems to be more common in children with renal conditions being treated with either rhGH or IGF-I (Guy *et al.* 1987; Koller *et al.* 1997) than in children being treated for other indications. The incidence in renal disease outwith rhGH treatment is higher than in the general population (Chang *et al.* 1992). It is a recognised but rare complication of rhGH treatment, and if children receiving rhGH treatment complain of headaches or blurred vision, examination, and in particular, fundoscopy is mandatory.

Renal bone disease or renal osteodystrophy is a frequent problem in children on dialysis. Renal osteodystrophy may prevent a good response to rhGH, or alternatively increased growth rates due to rhGH may worsen renal osteodystrophy. One child was

withdrawn because of worsening renal osteodystrophy, however as the growth response to rhGH was poor, it seems unlikely to be the cause. The effects of rhGH on renal bone disease are discussed in more detail in Chapter 10.

Conclusion

In summary, one year of rhGH increased the short term growth rate in children on longterm dialysis, with children on peritoneal dialysis showing a marginally better response than those on haemodialysis. The response in both groups was less than that seen in the CRI and transplanted groups. The children who showed the greatest improvement in HV were those who were growing most slowly prior to treatment.

Table 5.1 Patient details in the dialysis groups

	Peritoneal Dialysis	Haemodialysis	Peritoneal Dialysis	Haemodialysis
	<i>Prepubertal</i>	<i>Prepubertal</i>	<i>Pubertal</i>	<i>Pubertal</i>
Number (girls)	14 (6)	8 (1)	2 male	1 male
Age (years)	7.9 (3.5 - 16.9)	10.3 (3.8 - 16.5)	15.7, 12.3	15.5
Pubertal Stage			PH3 0808, PH2 0404	PH3 0808
<u>Aetiology</u>				
Congenital Structural	5	5		
Glomerulonephritis	5		1	
Reflux Nephropathy	1	3		1
Interstitial Nephritis	1		1	
Vascular	2	1		

Table 5.2 Growth data for the prepubertal peritoneal dialysis patients in the year before and the year of rhGH treatment

	<u>Day 1</u>	<u>1 Year</u>	<i>n=9</i>
Height SDS	-3.1 (0.6)	-2.7 (0.7)	<i>p = 0.001</i>
	<u>Pre rhGH</u>	<u>During rhGH</u>	
HV	5.0 (1.6)	7.5 (1.6)	<i>p = 0.001</i>
HVSDS	-0.6 (2.1)	2.5 (3.8)	<i>p = 0.01</i>
Δ height SDS	-0.2 (0.4)	0.4 (0.2)	<i>p = 0.007</i>

Table 5.3 Adverse events during rhGH treatment in the prepubertal peritoneal dialysis group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
1	4	Peritonitis	Resolved	None
1	8	Worsening renal bone disease	rhGH discontinued at 11.2 mths	Possibly related
3	6	Convulsions	Stable on anticonvulsants	Thought to be unrelated
4	2	Painful limp	Resolved	Possibly related
8	4 days	Hypertension and right facial palsy, followed by left facial palsy 3 weeks later	Resolved	Unlikely
8	9	Peritonitis	Resolved	None
8	12	Peritonitis	Resolved	None
9	3	Raised intracranial hypertension	Resolved	Thought to be related
10	9	Priapism	Resolved	Patient withdrawn
13	1	Bilateral hydrocoeles requiring surgery	Resolved but patient required temporary haemodialysis	Unlikely
13	2	Peritonitis	Patient transferred to haemodialysis	None

Table 5.4 Growth data for the prepubertal haemodialysis patients in the year before and the year of rhGH treatment

	<u>Day 1</u>	<u>1 Year</u>	<i>n=6</i>
Height SDS	-3.3 (1.1)	-3.1 (1.2)	<i>p</i> = 0.47
	<u>Pre rhGH</u>	<u>During rhGH</u>	
HV	3.9 (2.7)	5.5 (1.9)	<i>p</i> = 0.27
HVSDS	-2.4 (2.0)	-0.5 (3.1)	<i>p</i> = 0.32
Δ height SDS	0.0 (0.3)	0.1 (0.3)	<i>p</i> = 0.71

Table 5.5 Adverse events during rhGH treatment in the prepubertal haemodialysis group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
3	3	Ankle pain	Resolved	Possibly related
9	8	Headaches and blurred vision - no cause found	Resolved	Possibly related - rhGH continued

Table 5.6 Growth data for all of the prepubertal dialysis patients in the year before and the year of rhGH treatment

	<u>Day 1</u>	<u>1 Year</u>	<i>n=15</i>
Height SDS	-3.0 (0.9)	-2.8 (1.0)	p < 0.001
	<u>Pre rhGH</u>	<u>During rhGH</u>	
HV	4.6 (2.3)	6.5 (1.8)	p < 0.001
HVSDS	-1.3 (2.1)	1.1 (3.8)	p < 0.001
Δ height SDS	0.0 (0.3)	0.3 (0.3)	p = 0.03

Table 5.7 Growth data for the pubertal dialysis patients in the year before and the year of rhGH treatment

	<u>Day 1</u>	<u>1 Year</u>
Height SDS	-2.6, -1.0, -4.8	-1.3, -1.0, -4.2
	<u>Pre rhGH</u>	<u>During rhGH</u>
HV	8.4, 0.4, 5.0	13.1, 6.4, 6.4
HVSDS	2.7, -5.9, -0.7	11.5, 0, 4.2

Table 5.8 Adverse events during rhGH treatment in the pubertal dialysis group

<u>Patient Number</u>	<u>Time since starting rhGH (Mths)</u>	<u>Event</u>	<u>Outcome</u>	<u>Relationship to RhGH</u>
1	10 days	Inguinal hernia requiring surgical repair	Resolved	Unlikely
1	3	Umbilical repair requiring surgery	Resolved	Unlikely
2	5	Photosensitive facial rash	Resolved	Unlikely

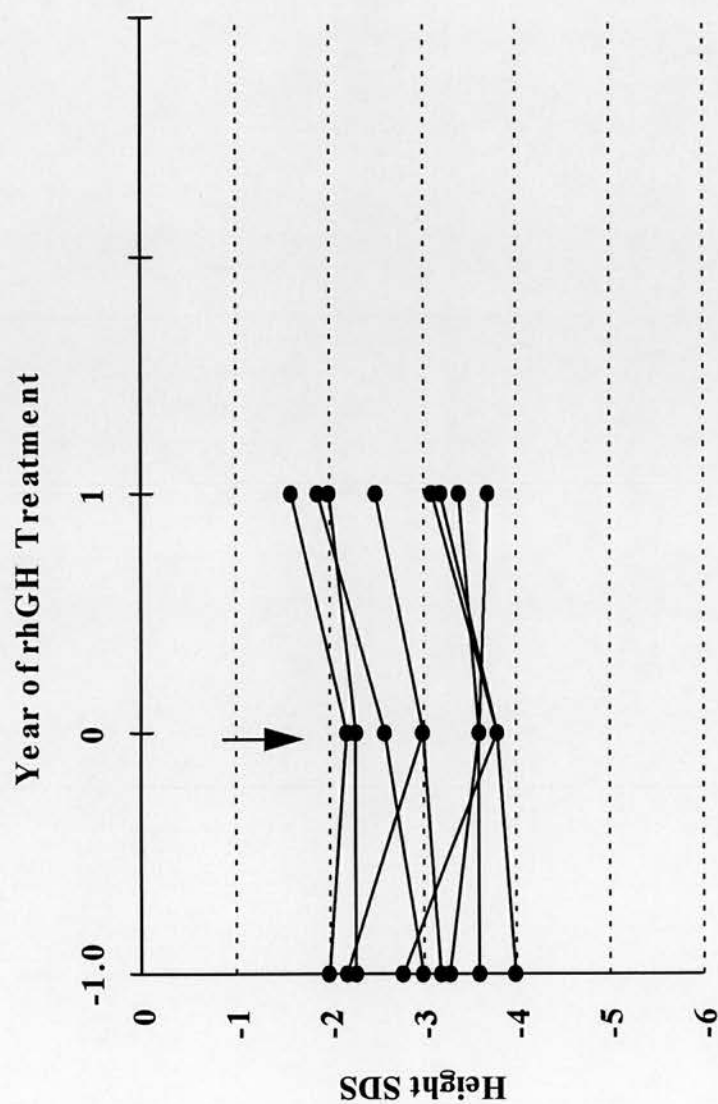


Figure 5.1 Individual height SDS measurements in the prepubertal peritoneal dialysis group 1 year before, at the time of starting rhGH (↓) and after 1 year of treatment.

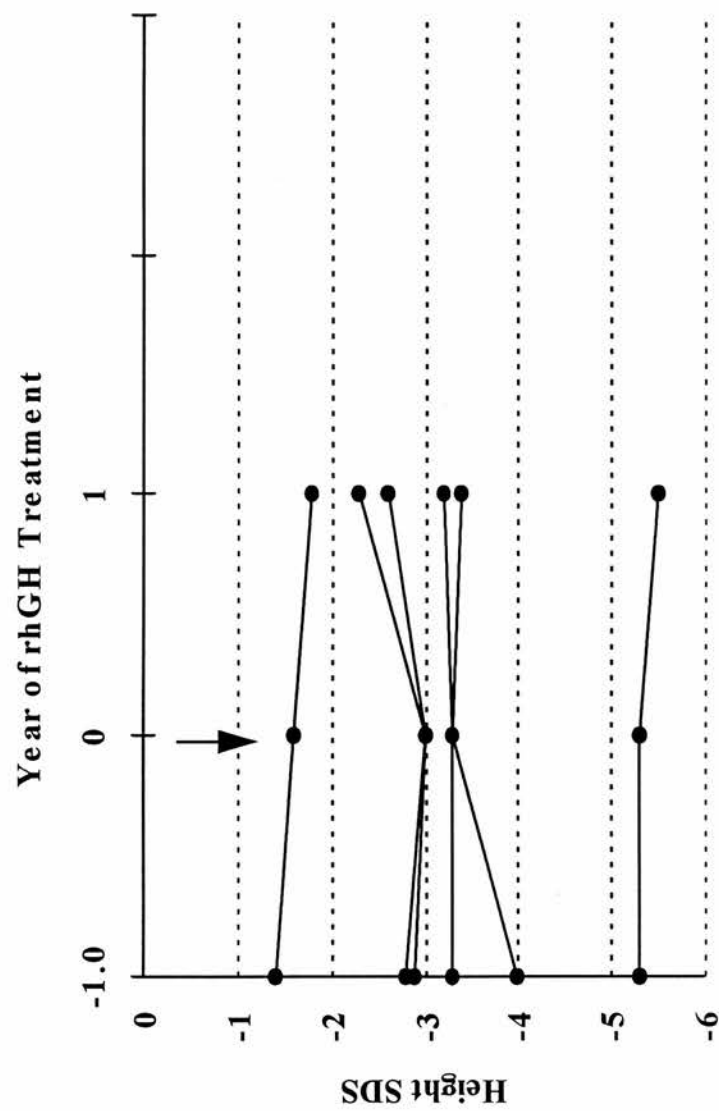


Figure 5.2 Individual height SDS measurements in the prepubertal haemodialysis group 1 year before, at the time of starting rhGH (\downarrow) and after 1 year of treatment.

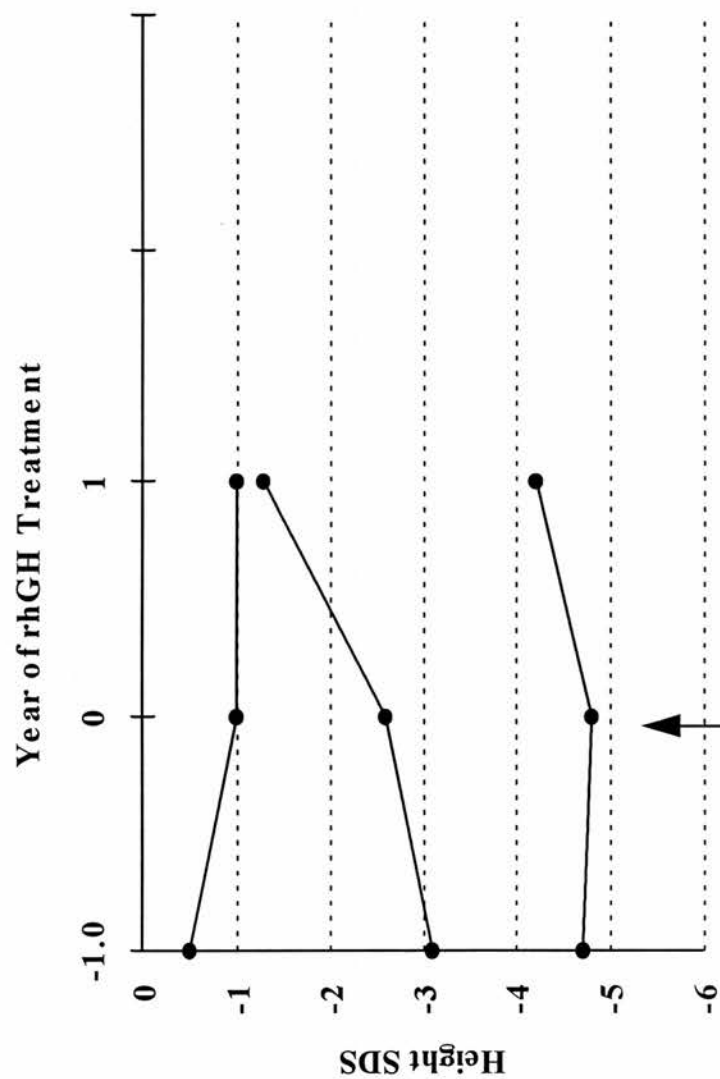


Figure 5.3 Individual height SDS measurements in the pubertal dialysis group 1 year before, at the time of starting rhGH (↑) and after 1 year of treatment.

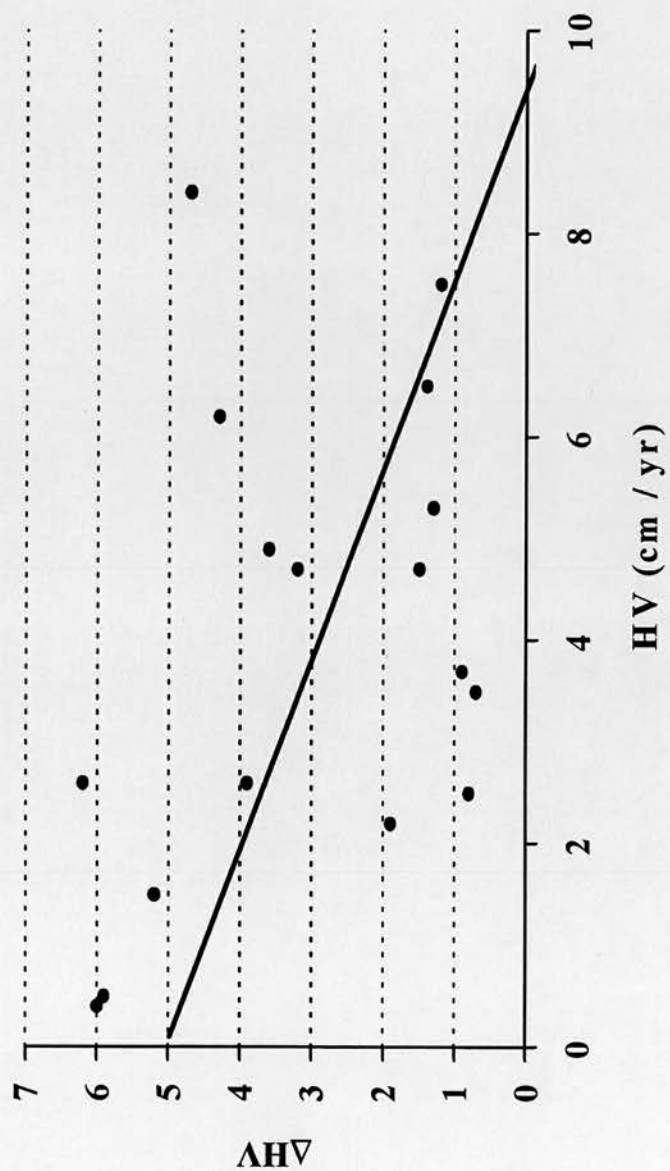


Figure 5.4 The increase in HV (Δ HV) in cm/yr during the first year of rhGH treatment in all of the dialysis children ($n=18$) plotted against HV in cm/yr at baseline, $r = -0.568$, $p = 0.014$.

Chapter 6: Randomised Controlled Trial of Recombinant Human Growth Hormone in Prepubertal and Pubertal Renal Transplant Recipients

Introduction

Short stature persists in some children following renal transplantation, and preliminary studies have shown a beneficial effect of rhGH (van Dop *et al.* 1989; Rees *et al.* 1990). The safety of rhGH in this group is of paramount importance; improved growth at the expense of graft function is not acceptable. For this reason, this arm of the trial was conducted in a controlled fashion; children were randomised to rhGH or no treatment in the first year, with all receiving rhGH in the second year.

In view of the known effects of rhGH, its use post transplantation requires close monitoring, with particular emphasis on the effect of treatment on renal function (Hirschberg *et al.* 1989), the incidence of transplant rejection (Tyden *et al.* 1990), renal bone disease (Watkins 1996), and glucose metabolism (Sonksen *et al.* 1967).

The children in the transplant group were older than the CRI and dialysis patients, and a greater percentage were approaching or were in the early stages of puberty. The effect of rhGH on the timing and duration of puberty are not known, therefore patients were stratified according to pubertal stage at entry into the trial.

Some of the children remained on rhGH therapy after the trial was complete and follow up data for these children are also reported.

Methods

Twenty-two children with renal transplants were enrolled into a 2 year open-labelled, prospective and controlled trial of the use of rhGH. Patients were assigned to prepubertal and pubertal groups, then subsequently randomised to rhGH (**treatment group**) or no rhGH treatment (**control group**) in the first year of the trial, with all children receiving rhGH in the second year (Figure 6.1). Six children continued on

rhGH treatment after completion of the trial; data from these children are also reported (**follow up data**).

The children were seen on day 1, day 8, and then at 3 monthly intervals for 2 years. On each occasion, height, weight, blood pressure and blood biochemistry including urea, creatinine, calcium, phosphate, alkaline phosphatase (ALP), intact parathyroid hormone (PTH), glucose, insulin and glycosylated haemoglobin (HbA1c) were checked.

Renal function was monitored every 6 months by clearance of inulin. A rejection episode was defined as an episode of transplant dysfunction treated with 3 days of high dose oral prednisolone. The diagnosis was made by the managing clinician. The children who remained on rhGH were monitored thereafter at 3 monthly intervals.

Children were considered eligible for entry to the trial if they fulfilled the general entry and exclusion criteria outlined in Chapter 2. In addition, the patients had received their transplants more than 1 year previously, and had a minimum calculated GFR of 20 ml/min/1.73m² (Schwartz *et al.* 1976).

Immunosuppression consisted of azathioprine (60 mg/m²), prednisolone (approximately 10 mg/m² on alternate days) and cyclosporin A. The dose of cyclosporin A was adjusted to maintain plasma trough levels between 50 and 150 ng/ml.

Patients

Fifteen prepubertal (2 girls) and 7 pubertal patients (2 girls) were enrolled and randomised to receive either rhGH or no rhGH in the first year. Patient details are given in Table 6.1. Height velocity and HVSDS during the year before the trial are given in Table 6.2; height SDS at baseline is given in Tables 6.3.

The mean time since transplantation was 6.5 (2.0 - 11.8) years. Prednisolone dose was 8.9 (0 - 20.1) mg/m² on alternate days in the prepubertal and 8.2 (0 - 15.2) in the pubertal group, equivalent to a daily dose of 0 - 10 mg/m² of prednisolone. Nine prepubertal and 4 pubertal children were randomised to receive rhGH in the first year (treatment group); all children received rhGH in the second year.

Follow up Data

Fourteen children were followed up for 2 years; 3 for 3 years, 2 for 5 years and 1 child was treated for 6 years. Height velocity and calculated GFR were measured during this time. In addition, the first year of rhGH treatment in all patients was compared with the growth rate during the preceding year. Age, pre-treatment HV and height SDS, GFR and steroid dose were correlated with the increase in HV and height SDS during the first year of treatment. In view of the older age of the patients, HV during the study was used in preference to HVSDS.

Statistical Analysis

Results are expressed as mean (range) or mean (SD). Within and between group results were compared using the paired or unpaired Students *t*-test respectively. Comparison of change within the treatment and control groups was made by calculating and comparing the mean (SE) change. Frequencies were compared using the chi-squared test. Analysis of multiple results within the same group over time was performed by ANOVA. Correlation's were performed using Pearson's correlation coefficient, and regression by both single and multiple linear regression analyses. Statistical significance is assumed with a p value of < 0.05.

Results

Treatment Group (9 prepubertal, 4 pubertal)

Two prepubertal children stopped rhGH; one child returned to dialysis after 11 months, the other developed glucose intolerance after 9 months of treatment. Two children stopped treatment during the second year: one prepubertal child had started the trial with poor graft function and received a second graft after 18 months of rhGH; the other pubertal child had an increase in creatinine and elected to stop rhGH treatment at the beginning of the second year. Episodes of transplant dysfunction are shown in Table 6.4.

Control Group (6 prepubertal, 3 pubertal)

One pubertal child was withdrawn after 18 months because of a poor response to 6 months of rhGH therapy.

Growth

Prepubertal Patients (n=15)

During the first year of the study HV and HVSDS were greater in the treatment than control group, Table 6.2. Mean (SE) Δ height SDS during the first year of study was 0.6 (0.1) in the treatment group and -0.3 (0.2) in the control group, $p < 0.001$, Table 6.3.

In the treatment group there was no difference between Δ height SDS in the first and second years of treatment; 0.6 (0.1) vs. 0.4 (0.2), $p = 0.34$. Height velocity and HVSDS were lower in the second year compared to the first year of treatment, Table 6.2. The increase in height SDS during the second year in the treatment group was greater than that in year 1 in the control group [0.4 (0.2) vs. -0.3 (0.2), $p = 0.003$], but HV and HVSDS were not significantly different; $p = 0.16$ and 0.09 respectively. In the control group, HV, HVSDS and Δ height SDS were greater in the second year on rhGH treatment than in the first year, Tables 6.2 & 6.3.

Pubertal Patients (n = 7)

The results in the pubertal group were similar to those in the prepubertal group, however the number of children in the treatment and control groups was small. During the first year of the trial, HV and Δ height SDS were significantly greater in the treatment than control group; the corresponding p value for HVSDS was $p = 0.06$, Tables 6.2 & 6.3.

In the treatment group, HV, HVSDS and Δ height SDS remained above pre-treatment levels in the second year of the study, but were lower than the respective values in the first year of the trial. Comparing the first and second year data in the control group, HV, HVSDS and Δ height SDS were greater in the year 2 than in year 1, but the values were not statistically significant, Tables 6.2 & 6.3.

Pooled Follow-Up Data for All Prepubertal Children during rhGH Treatment

When data are pooled for all 15 prepubertal children during their first year of rhGH treatment, HV, HVSDS and Δ height SDS all increased significantly compared with the year before treatment, Table 6.5, Figures 6.2 and 6.3. Height SDS at each time point is shown in Figure 6.4.

Ten prepubertal children received rhGH for at least 2 years. In these children height SDS increased from -3.4 (-5.8 to -1.9) on day 1, to -2.4 (-4.4 to -1.2) after 2 years, $p=0.002$ (Figure 6.2). Height velocity was 3.9 (0.8 - 6.9) in the year before treatment; 8.2 (3.0 - 10.7) in the first year, $p<0.0002$ vs. year before; and 6.1 (0.1 - 8.8) during the second year, $p=0.012$ compared to the year before treatment (Figure 6.3). The mean gain in height was 14.8 (3.1 - 18.5) cm in 2 years. Three children received 3 years of rhGH; mean height gain was 22.1 (21.8 - 22.4) cm in 3 yrs. One child received rhGH for 5 years and another for 6 years (Figure 6.4); HVSDS remained above pre-treatment values during this time.

Pooled Follow-Up Data for All Pubertal Children during rhGH Treatment

Considering all 7 pubertal children, HV, HVSDS and Δ height SDS all increased significantly when compared to the year before treatment, Table 6.5 and Figures 6.5 and 6.6. Height SDS at each time point is shown in Figure 6.7. Five of the 7 patients completed the study, but elected to stop treatment. Four children received 2 years of rhGH: HV was 5.2 (3.5 - 8.1) before treatment; 9.3 (5.3 - 11.7) in the first year; and 5.8 (4.1 - 8.7) in the second year, values very similar to the prepubertal group. The average height gain during 2 years of rhGH was 15.0 (9.5 - 19.3) cm.

Regression Analysis

There was no significant difference in the increase in height SDS and HV in the first year of treatment between the prepubertal and pubertal groups. Combining the results from all 22 transplanted children, HV in the year before treatment was correlated with age ($r = -0.417$, $p = 0.05$) as was HV during rhGH treatment ($r = -0.463$, $p = 0.03$).

Multiple regression analysis revealed prednisolone dose ($p = 0.028$), Figure 6.8, and age ($p = 0.049$) as the strongest negative predictors of Δ height SDS during rhGH treatment. Using single regression analysis, HV during treatment was positively correlated with GFR ($r = 0.429$, $p = 0.016$), but this relationship became non-significant when the effects of age and prednisolone dose were taken into account. There was no significant relationship between Δ height SDS and height SDS on day 1 ($r = 0.034$) or between Δ height SDS and HV ($r = 0.265$) before treatment.

The increase in HV during the first year of rhGH treatment was unrelated to pre-treatment HV ($r = -0.265$, $p = 0.23$), to GFR ($r = 0.223$), to age ($r = -0.199$) and to dose of prednisolone ($r = -0.102$).

Weight for Height

At the start of treatment, all children had a WFH > 100%. The two lowest values (105% and 108%) were in the two children who received no prednisolone therapy. Percentage WFH decreased during the first year of treatment from 133 (105 - 171)% at the start of treatment, to 125 (99 - 152)% at 6 months ($p < 0.001$), and 122 (96 - 152)% at 1 year ($p < 0.001$). WFH remained significantly reduced after two years 120 (88 - 168)% ($p = 0.016$).

Renal Function

Results for the prepubertal and pubertal children have been combined. Serum creatinine in the treatment and control groups did not differ at baseline ($p = 0.46$), Table 6.6. The mean (SE) change in creatinine during the first year appeared greater in the treated group [30.1 (11.7) $\mu\text{mol/l}$] compared with the control group [3.8 (6.2)], but the difference was not statistically significant ($p = 0.11$). Creatinine at 1 year was 146 (64 - 317) in the treated group ($p = 0.013$ vs. day 1), and did not increase further during the second year of treatment [146 (70 - 402) at 2 years]. Urea decreased slightly after 1 week of rhGH, but was unchanged thereafter (data not shown).

Results for the prepubertal and pubertal children have been combined; GFR at baseline was no different in the treated and control groups, $p = 0.12$, Table 6.6. The mean change in GFR during the first year was 9.9 (5.4) mls/min/1.73m^2 in the treated group ($n = 12$) and -1.6 (7.6) in the control group, ($n = 9$), $p = 0.22$. One child was withdrawn during the first year because of graft failure. Setting his GFR to zero at the 1 year visit does not significantly alter the change in GFR during the first year; 7.2 (6.0), $p = 0.36$.

More detailed data relating to individual GFR values during rhGH treatment are shown in Chapter 7. The mean (range) values for creatinine and GFR for all children during rhGH treatment are shown in Table 6.7. For those children withdrawn due to glucose intolerance and poor response, the last available GFR has been used for the end of treatment value. Where graft failure occurred, a value of zero was used.

GFR increased significantly during rhGH treatment: 49 (13 - 117) on day 1 and 58 (14 - 133) mls/min/1.73m² after 6 months, n = 22, p = 0.01. Taking only those children who completed a full year of rhGH, GFR remained elevated at one year; 51 vs. 59, (n = 19, p = 0.04). However if a value of zero is included for the child who lost his graft, then the change in GFR becomes non-significant; (n = 20, p = 0.12). There was no further change in GFR during the second year of rhGH treatment. Calculated GFR in the children who continued on treatment after the study was complete, did not change (data not shown).

Presumed Rejection Episodes

In the first year of the trial there were 8 presumed rejection episodes in 5 of the 13 patients in the treatment group and 4 in 2 of 9 patients in the control group (p >0.1). In the control group there was no difference between the number of episodes in the first and second years of the trial; 4 in 2 of 9 patients vs. 4 in 2 of 9. Combining the data from all 22 children, there were 12 episodes in 22 patients in the first year of treatment, which was no different from the year before rhGH (15 in 22 patients), p>0.1. Further information relating to these episodes of transplant dysfunction is given in Table 6.4.

Adverse Events

In addition to the transplant dysfunction described above, two other adverse events were reported. A 10 year old girl with a renal transplant had an elevated fasting glucose, insulin, and HbA1c after 9 months of rhGH. These returned to normal when rhGH was stopped. Several years before, whilst on dialysis, she had pancreatitis and required a partial pancreatectomy. Glucose tolerance tests before and after withdrawal from the trial were normal. She went on to develop insulin dependent diabetes, and chose to re-start rhGH therapy once established on insulin replacement.

A 13 year old boy in the transplant control group developed worsening of a pre-existing idiopathic scoliosis during rhGH which required surgical correction the following year.

Discussion

This is one of the few controlled trials of rhGH in children with renal transplants to date. Growth hormone improved growth in both the prepubertal and pubertal transplant patients. The growth response was equal in the two groups, and improved growth persisted during the second year of treatment. Comparing the control and treatment groups, there was no apparent adverse effect on graft function.

The best response to rhGH was seen in the youngest children and in those on the least steroid, with the strongest predictor being the dose of prednisolone ($p=0.029$). Growth in children with renal transplants, who are not on rhGH, has been correlated with age, GFR and also with steroid therapy (Guest *et al.* 1991; Tejani *et al.* 1993). Children on alternate day steroids grow better than those on daily steroids (Guest *et al.* 1991), and peak height velocity during puberty in patients with renal transplants is inversely correlated with steroid dose (Schaefer *et al.* 1990), so the relationship between response to rhGH and steroid dose is not surprising. In another post transplant study (Ingulli *et al.* 1993), change in height SDS was greater than 0.4 after one year of rhGH in 5 of 5 children on no steroid and one child on alternate day steroids, but only -0.2 (-0.6 to 0.3) in 11 children on daily steroids. Mean creatinine was the same in both groups. All of our patients were receiving alternate day steroids.

Whether rhGH benefits final height, particularly if it is not started until puberty, has yet to be determined. In renal failure, puberty is delayed and the magnitude of the pubertal growth spurt attenuated (Schaefer *et al.* 1990). Puberty would seem to be an appropriate time to use rhGH, to induce or mimic the endogenous growth spurt, but there is concern that treatment might shorten the duration of the pubertal growth spurt (Darendililer *et al.* 1990). Some reports have suggested that this may be the

case in children with renal transplants who receive rhGH (Bartosh *et al.* 1992; Benfield *et al.* 1993), whilst others have demonstrated an increase in height during adolescence (Fine *et al.* 1992; Hokken-Koelega *et al.* 1994d; Maxwell *et al.* 1996d). There was no undue advancement in bone age in this study (data not shown), nor in other reported studies (Hokken-Koelega *et al.* 1994d). The increase in height SDS was similar in the prepubertal and pubertal treatment groups in our study, with little or no change in height SDS in either of the control groups. Longer follow up however will be necessary to determine if there is a positive effect on final adult height.

Preliminary final height data are becoming available. Janssen assessed final height in 17 renal transplant patients who had received rhGH; the median final height SDS of -1.8 for males was significantly higher than controls. Six of the 17 patients had a target height within the predicted range (Janssen *et al.* 1997). Broyer presented data from 13 children with renal transplants who had received rhGH. Height SDS was -3.5 at the time of starting rhGH and final height SDS was -1.8. (Broyer M, data presented at 1st meeting of the International Paediatric Transplantation Association, Venice, August 2000).

Rees has published final adult height data from 6 prepubertal and 6 pubertal renal transplant recipients. In the prepubertal patients height SDS increased from -3.3 to -2.9 after 2.7 years of rhGH, and was -3.0 at final height. In the pubertal patients height increased from -3.4 to -2.6 after 1.4 years of rhGH treatment, and was -2.5 at final height (Rees *et al.* 2000). The German group have also presented preliminary final height data in 38 children with renal transplants who received rhGH treatment. Height SDS before treatment was -3.2. An increase of +1.5 SD was seen in the males and of +1.2 was seen in the female patients during treatment. This compares with 50 control patients who had a mean height SDS of -1.5, and whose height SDS changed by -0.7 in the boys and -0.5 in the girls during an equivalent time period (Haffner D, data presented at the European Society of Pediatric Nephrology, Helsinki, June 2000). From the preliminary evidence available, rhGH treatment would appear to

have a beneficial effect on final height, but further data are required before any firm conclusion can be drawn.

One of the patients developed glucose intolerance during the study. This is a recognised complication of acromegaly (Sonksen *et al.* 1967). Patients with renal disease have peripheral resistance to the actions of insulin (Mak and DeFronzo 1992), which is aggravated in transplanted patients by the use of corticosteroids (Fennell *et al.* 1983). Glucose intolerance during rhGH treatment post transplantation has been reported previously (Ingulli *et al.* 1993). Our patient had undergone a partial pancreatectomy which may have been a contributing factor. For the group as a whole, the increase in fasting glucose and insulin were transient and during two years of rhGH there was no increase in HbA1c. In the long term there would appear to be no adverse effect on glucose tolerance. This issue is discussed further in Chapter 10.

There are several possible mechanisms whereby rhGH or its mediator insulin-like growth factor-I (IGF-I), might affect graft function: rhGH increases renal plasma flow and GFR in adults with normal renal function (Hirschberg *et al.* 1989; Hammerman *et al.* 1989); there are known interactions between rhGH and the immune system which could potentially cause an increase in the rate of rejection episodes (Kelley *et al.* 1990); and lastly rhGH could have a direct effect on the kidney, as there are growth hormone receptors on mesangial cells, and receptors IGF-I on proximal tubular and mesangial cells (Arnquist *et al.* 1988). Because of the relationship between rejection episodes and GFR, it is very difficult to study the effects of rhGH on these parameters in isolation. The effects of rhGH on renal function are discussed at length in Chapter 7.

In renal impairment, an increase in GFR might hasten the progression of decline in GFR, the so-called hyperfiltration theory (Brenner *et al.* 1985). There is no evidence that rhGH adversely affects GFR in children with CRI (Tonshoff *et al.* 1992; Maxwell *et al.* 1995), but few studies of rhGH in renal transplantation have formally assessed GFR. Most report changes in creatinine or calculated GFR, and an analysis of these

trials has been inconclusive (Chavers *et al.* 1995). The data presented here suggest that 2 years of rhGH treatment does not adversely affect graft function.

However two children were withdrawn from the study because of a deterioration of renal function. For one patient there was suspicion of non-adherence to immunosuppression, whilst the other child had an acute rejection episode after 6 months of treatment. His GFR at 1 year was little changed from baseline, but 1 week later he had a further increase in creatinine and was withdrawn from the study. A third child received a second renal transplant after 18 months of rhGH. His renal function had shown a gradual deterioration before and during the trial. The small number of patients, the variability of the clinical course post transplantation, and the expected decline in graft function over time hamper interpretation of the data.

There was no apparent untoward effect of rhGH on the incidence of acute rejection. Some reports suggest rhGH does not affect acute rejection (Rees *et al.* 1990; Fine *et al.* 1992; Hokken-Koelega *et al.* 1994d, 1996), while others suggest that it does (Tyden *et al.* 1990; Schwartz and Warady 1992; Guest *et al.* 1998). Biopsy proven acute rejection has been documented in several children at varying intervals after starting rhGH (Tyden *et al.* 1990; Johansson *et al.* 1990; Schwartz and Warady 1992; Ingulli *et al.* 1993). None of these were controlled trials. One study suggests that the risk of rejection is increased by rhGH, but only in children who have had rejection episodes prior to treatment (Guest *et al.* 1998). During the first year of rhGH treatment, 7 of our patients had 12 presumed rejection episodes; all but one had had presumed rejection episodes in the previous year.

The incidence of rejection episodes in the study is higher than other studies. This is explained partly by the fact that these were presumed and not biopsy proven episodes, and therefore likely to be an overestimation. Several patients had chronic rejection at the start of treatment, and other studies report increases in creatinine during rhGH when there is biopsy-proven chronic rejection (Johansson *et al.* 1990; Van Dop *et al.* 1992; Benfield *et al.* 1993). It is difficult to determine if rhGH has decreased GFR, if

there has been an increase in muscle bulk, or if this is the natural progression of chronic rejection. A recent study of mixed lymphocyte cultures using lymphocytes from paediatric renal transplant recipients and donor cells, showed that overall, the addition of rhGH to the culture had little effect, although 3 patients had an augmented response. These 3 patients had biopsy proven chronic rejection (Benfield *et al.* 1996).

An ongoing study is addressing the issue of rhGH and rejection in patients with renal transplants. Fine is conducting a randomised controlled study which requires a renal biopsy at the start of the study before entry, and requires biopsy confirmation of rejection episodes (Fine RN, data presented at 1st meeting of the International Paediatric Transplantation Association, Venice, August 2000). Preliminary results in 38 children in the treatment group and 29 in the control group show no significant difference between the incidence of rejection in the two groups.

Conclusion

Recombinant human GH improves short term growth in prepubertal and pubertal children with renal transplants compared to controls. Height velocity during the second year of rhGH treatment remained above the baseline value in the prepubertal group. In both groups approximate height gain was 15 cm after 2 years of rhGH treatment. There was no increase in the incidence of rejection episodes. There was a significant increase in GFR during the first six months of treatment, but no change thereafter. With continued use of rhGH, HV decreases toward baseline, but the growth rate remains above pre-treatment values for up to 6 years .

Table 6.1 Patient details in the prepubertal and pubertal renal transplant groups

	Prepubertal	Pubertal
NUMBER (girls)	15 (2)	7 (2)
AGE (years)	13.0 (9.4 - 16.5)	15.2 (12.4 - 19.8)
GFR (ml/min/1.73m²)	51 (13 - 117)	48 (32 - 78)
AETIOLOGY		
Congenital structural	11	3
Glomerulonephritis	2	2
Congenital nephropathy		1
Cystinosis	1	1
HUS	1	

Table 6.2 Height velocity (HV) and height velocity SDS (HVSDS) in the prepubertal and pubertal transplant patients in the year before, and during the two years of the study. Results are shown for the treatment and control groups.

	HV (cm/yr)			HVSDS		
	Year Before	Year 1	Year 2	Year Before	Year 1	Year 2
<u>Prepubertal</u>						
Treatment group (n=9)	3.9 (1.7)	8.1 (2.7)	5.7 (2.9)**	-0.8 (1.8)	5.3 (5.0)	3.0 (5.8)**
Control group (n=6)	4.9 (1.8)	3.7 (1.5)	8.5 (1.6)**	-1.1 (3.0)	-1.6 (2.1)	3.0 (4.6)*
		$p < 0.005$			$p < 0.01$	
<u>Pubertal</u>						
Treatment group (n=4)	4.5 (2.5)	10.1 (1.2)	6.4 (2.1)	-2.8 (2.1)	5.5 (3.9)	3.6 (1.9)
Control group (n=3)	2.7 (1.6)	3.9 (2.3)	6.1 (4.7)	-3.0 (2.6)	-0.2 (1.0)	4.9 (5.9)
		$p < 0.005$			$p = 0.06$	

Mean (SD). The treatment and control groups are compared, as are first and second year results for each group.

** $p < 0.005$ * $p < 0.05$ vs. Year Before same group

Table 6.3 Height SDS at yearly intervals for the prepubertal and pubertal transplanted treatment and control groups.

Height SDS	- 1 Year	Day 1	Year 1	Year 2
<u>Prepubertal</u>				
Treatment group (n=9)	-3.4 (0.7)	-3.6 (1.0)	-3.0 (1.0)**	-2.6 (1.3)*
Control group (n=6)	-3.0 (0.7)	-3.0 (0.8)	-3.3 (1.0)	-2.8 (1.0) ^a
<u>Pubertal</u>				
Treatment group (n=4)	-2.1 (1.2)	-2.4 (1.4)	-1.9(1.3)*	-0.9 (0.4)
Control group (n=3)	-2.3 (0.1)	-2.6 (0.2)	-2.7 (0.5)	-2.3 (0.8)

Mean (SD)

Within the same groups, ** $p = 0.001$ vs Day 1, * $p = 0.02$ vs Day 1, ^a $p = 0.02$ vs Year 1

Table 6.4 Adverse events and presumed rejection episodes in the transplanted patients

	Time on rhGH (years)	Year 1 Adverse Events	Rejection Episodes	Year 2 Adverse Events	Rejection Episodes
Treatment Group					
Prepubertal					
1	2	-	-	-	-
2	2	-	-	-	-
3	2	-	-	-	-
4	2	-	-	-	-
5	0.75 * ¹	withdrawn (graft loss)	1		
6	0.75 * ¹	withdrawn (glucose intolerance)	-		
**7	2	-	-	decreased function	1
8	2	-	1	-	2
**9	1.5 * ²	decrease in function	3	withdrawn (2nd transplant)	2
Pubertal					
16	2	-	-	-	1
**17	2	decrease in function	2	-	2
18	2	-	-	-	-
19	1 * ¹	withdrawn (decreased function)	1		
Control Group					
Prepubertal					
10	1	-	-	-	-
11	1	-	-	-	-
12	1	-	-	-	-
13	1	-	-	-	-
14	1	-	2	worsening of scoliosis	1
15	1	-	-	-	-
Pubertal					
20	1	-	2	decrease in function	3
21	1	-	-	-	-
22	0.5 * ²	-	-	withdrawn (poor response)	-

*¹ withdrawn in year 1, *² withdrawn in year 2, **biopsy proven chronic rejection

Table 6.5 Growth data for the prepubertal and pubertal transplanted patients in the year before and during the first year of rhGH treatment

	Height SDS		HV (cm/yr)		HVSDS	
	Day 1	After 1 Year	Previous Year	Year 1	Previous Year	Year 1
Prepubertal (n=15)	-3.5 (1.0)	-2.9 (1.0)*	3.8 (1.6)	8.2 (2.3) *	-1.1 (1.9)	4.4 (4.8) *
Pubertal (n=7)	-2.5 (1.0)	-2.0 (1.0) ***	4.2 (2.2)	8.4 (3.6) ***	-1.7 (2.1)	5.2 (4.4) **

* $p < 0.001$, ** $p < 0.005$, *** $p < 0.05$ vs pre rhGH

Table 6.6 Renal function in the transplanted treatment and control groups

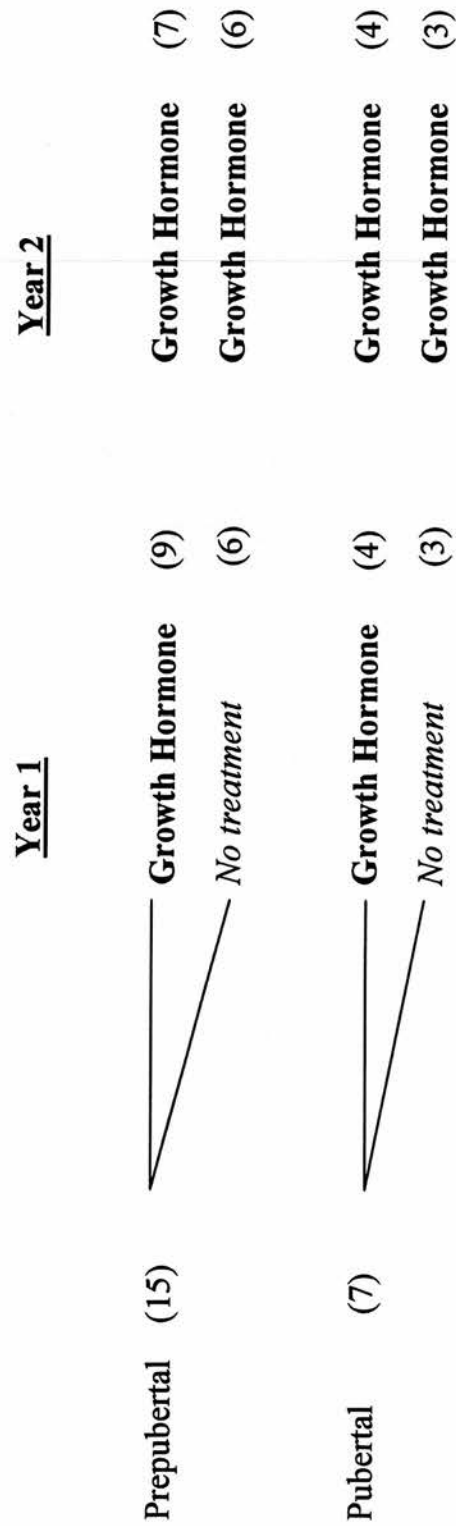
	Creatinine		GFR	
	Control (n = 9)	Treatment (n = 13)	Control (n = 9)	Treatment (n = 13)
Day 1	100 (49 - 152)	114 (46 - 220)	59 (19 - 105)	43 (18 - 70)
6 Months	101 (42 - 151)	134 (67 - 357)	66 (10 - 134)	53 (18 - 99)*
1 Year	103 (43 - 144)	146 (64 - 317)	58 (13 - 117)	49 (0 - 109)
ANOVA	$p = 0.42$	$p = 0.04$	$p = 0.43$	$p = 0.20$

* $p = 0.04$ vs. Day 1

Table 6.7 Renal function in all transplanted children during their first year of rhGH treatment

	Day 1	6 Months	1 Year	ANOVA
Creatinine (n=22)	110 (43 - 221)	123 (37 - 357)	129 (36 - 317)	$p = 0.06$
GFR (n = 22)	49 (13 - 117)	58 (14 - 133)	55 (0 - 113)	$p = 0.029$

Figure 6.1 Outline of the transplant arm of the BAPN study



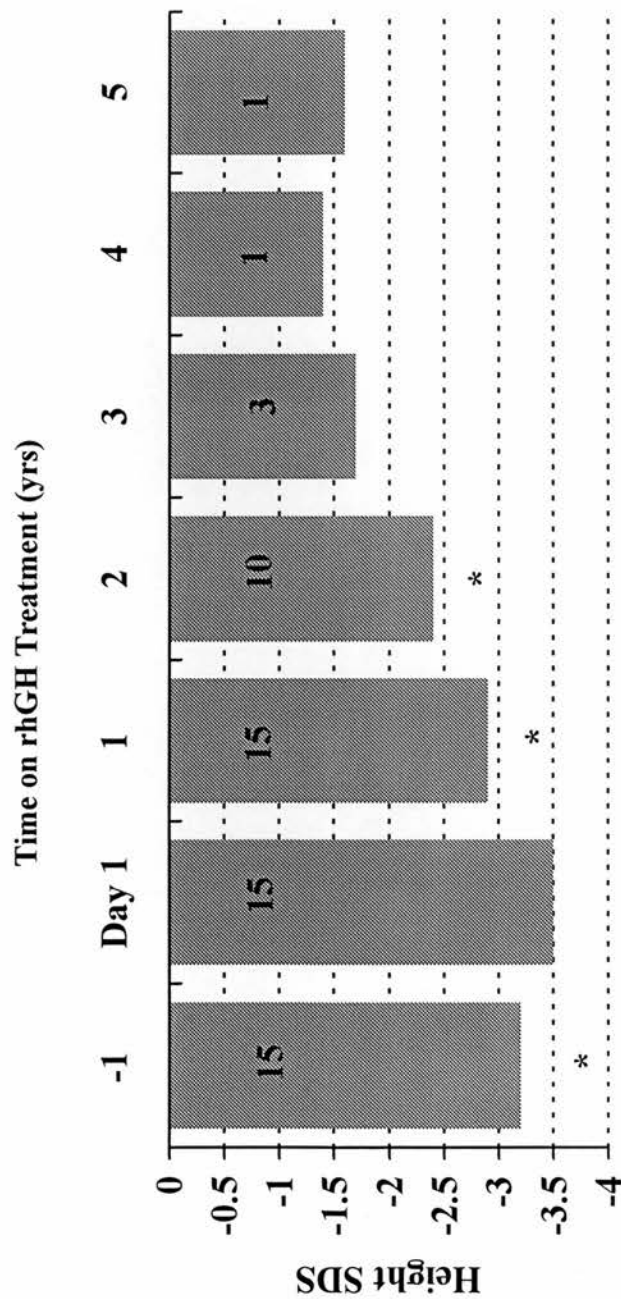


Figure 6.2 Mean height SDS in the prepubertal transplant patients 1 year before, at the start of the study, and at yearly intervals thereafter during rhGH treatment, * $p < 0.01$ vs day 1.

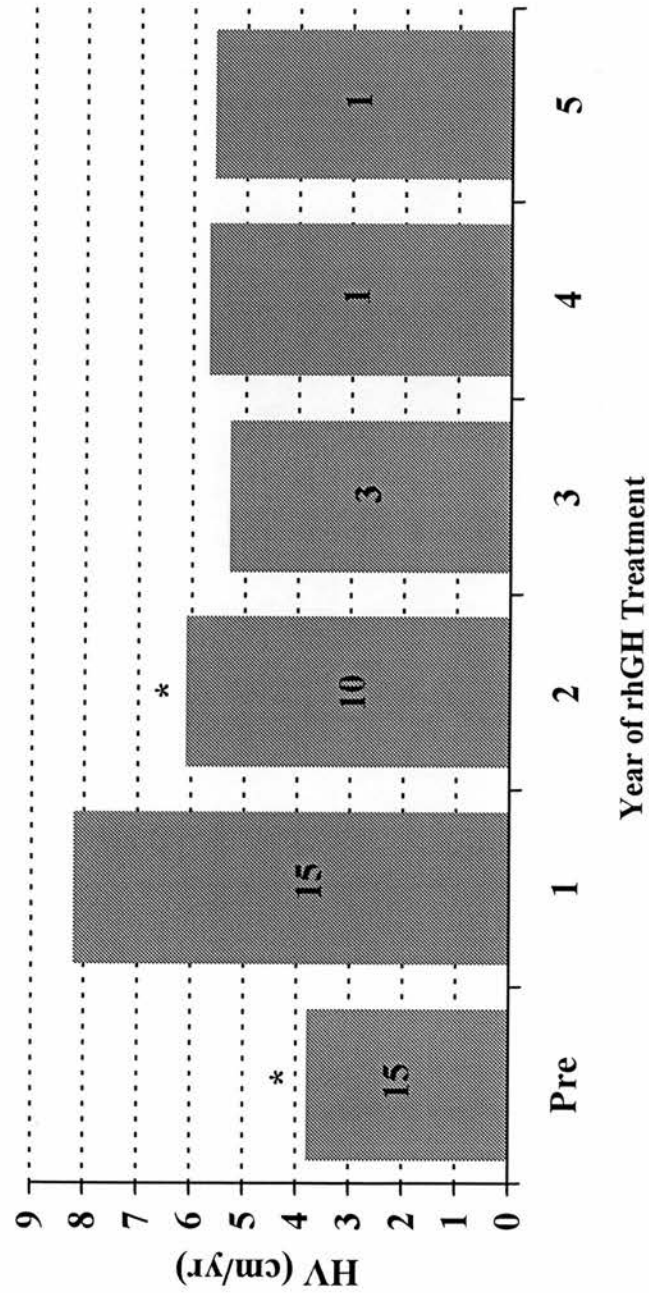


Figure 6.3 Mean height velocity (HV) in cm/yr in the prepubertal transplanted patients, in the year before, and during 5 years of rhGH treatment, * $p < 0.01$ vs first year of treatment.

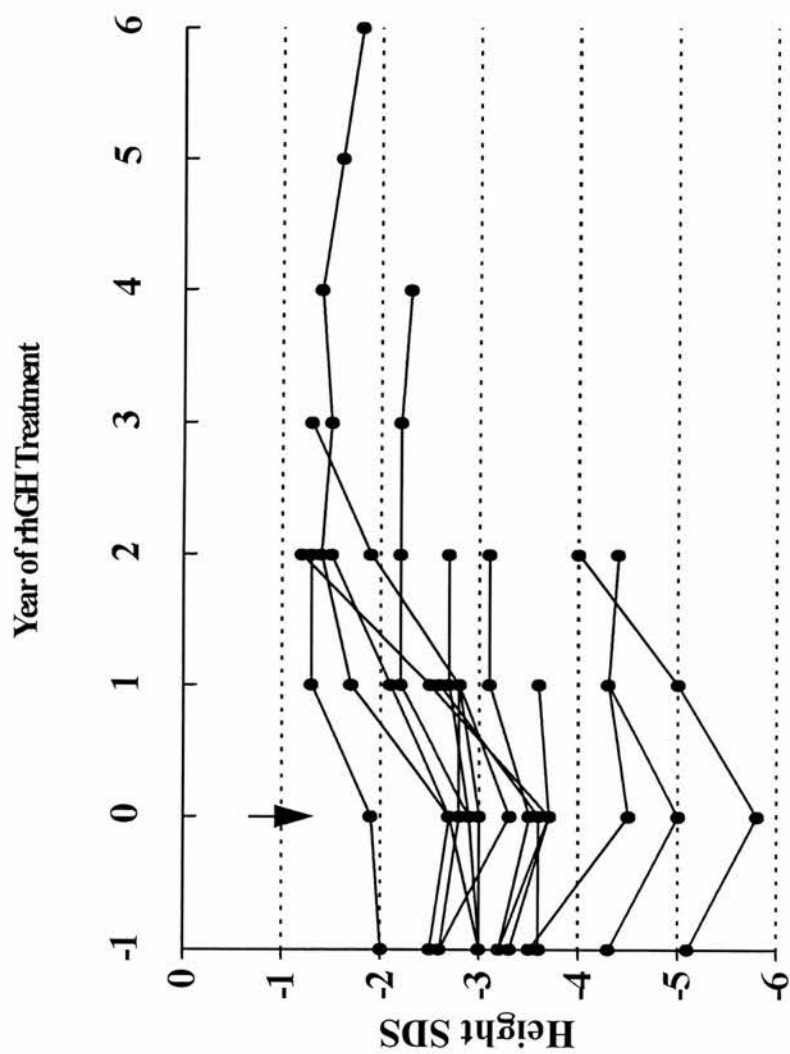


Figure 6.4 Individual height SDS measurements in the prepubertal transplanted patients 1 year before, at the start of the study (\downarrow), and at yearly intervals thereafter during rhGH treatment.

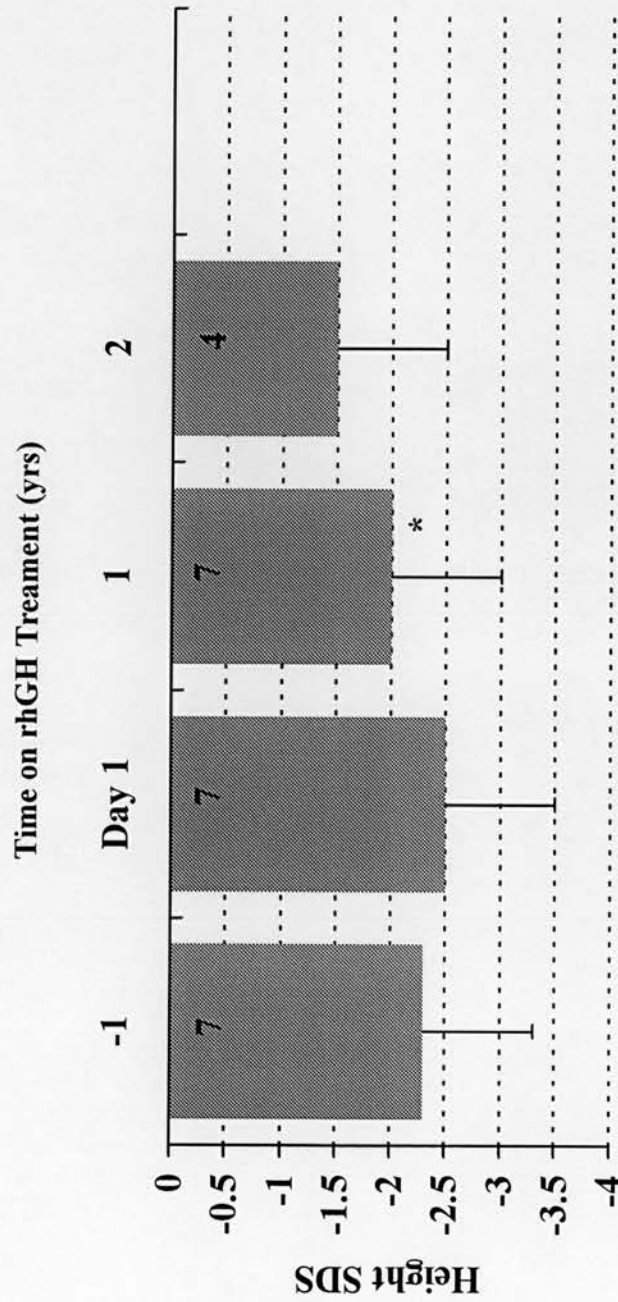


Figure 6.5 Mean (SD) height SDS in the pubertal transplanted patients 1 year before, at the start of the study, and at yearly intervals thereafter during rhGH treatment, * $p = 0.04$ vs day 1.

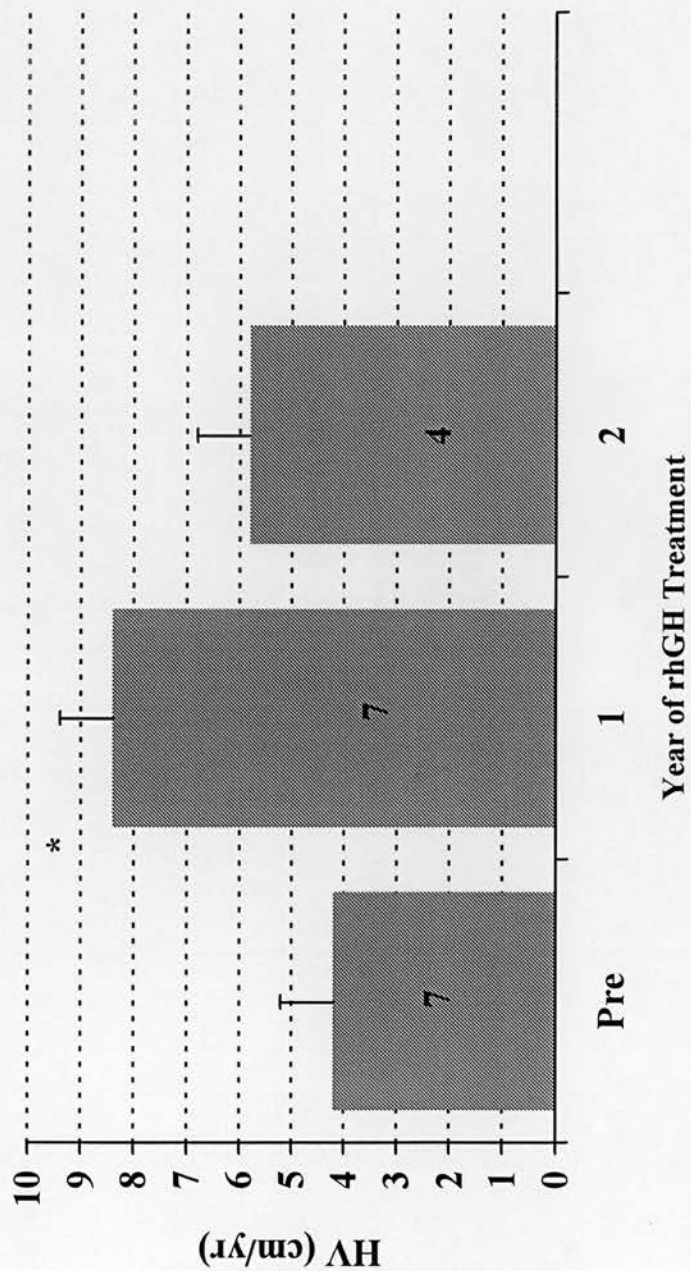


Figure 6.6 Mean (SD) height velocity (HV) in cm/yr in the pubertal transplanted patients in the year before the study, and during 2 years of rhGH treatment, * $p = 0.04$ vs year before treatment (*Pre*).

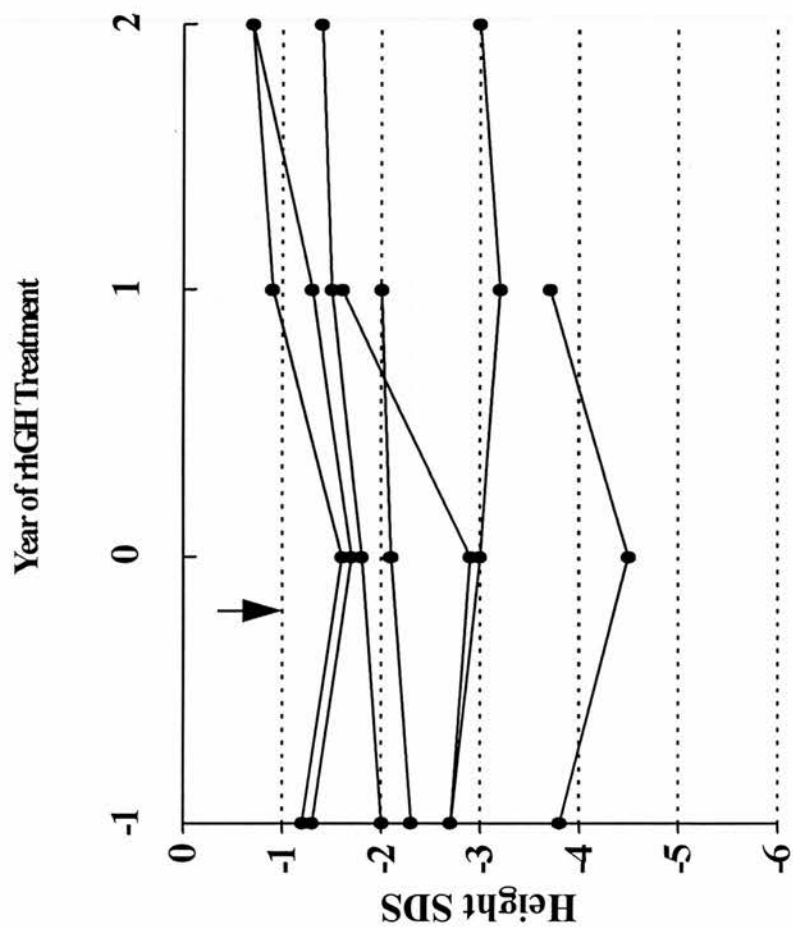


Figure 6.7 Individual height SDS measurements in the pubertal transplanted patients 1 year before, at the start of the study (\downarrow), and at yearly intervals thereafter during rhGH treatment.

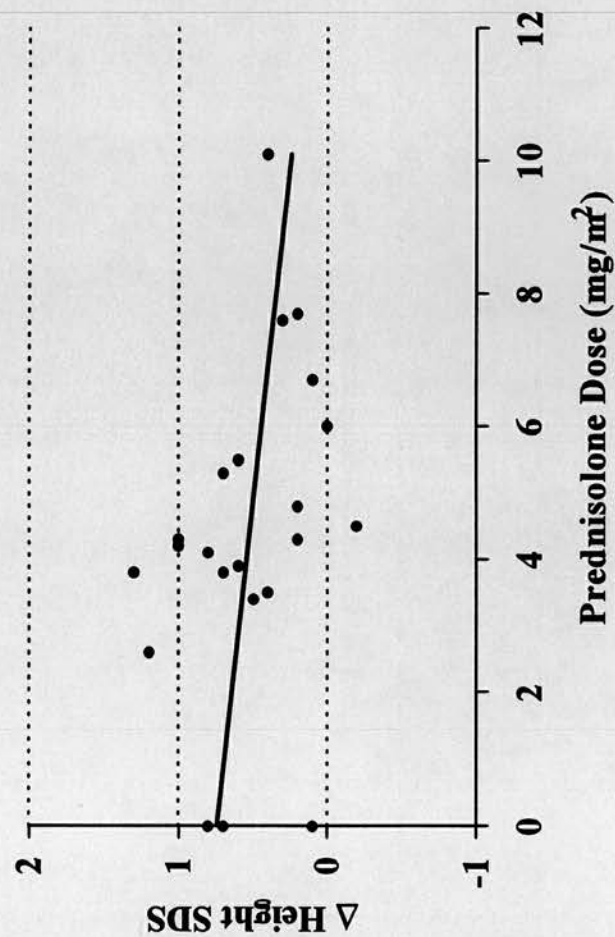


Figure 6.8 Increase in height SDS (Δ height SDS) during the first year of rhGH treatment plotted against the dose of prednisolone in all transplanted patients, $p = 0.03$.

Chapter 7: Effects Of Recombinant Human Growth Hormone On Renal Function in Chronic Renal Insufficiency and Following Renal Transplantation

Introduction

It has been known for some time that patients with acromegaly have large kidneys that hyperfilter (Ikkos *et al.* 1956; Gershberg *et al.* 1957). Recombinant human growth hormone, when given experimentally to adults with normal renal function, increases GFR and ERPF (Corvilian *et al.* 1962; Hirschberg *et al.* 1989; Christiansen *et al.* 1991). This effect occurs after several hours, at a time when GH levels have returned to normal, but levels of IGF-I are elevated (Parving *et al.* 1978; Hirschberg *et al.* 1989). Indeed administration of recombinant human IGF-I has the same effect (Guler *et al.* 1989; Hirschberg *et al.* 1993). Micropuncture studies in the rat demonstrate that this is due to an effect on glomerular haemodynamics (Hirschberg *et al.* 1991).

Growth hormone treatment in patients with CRI results in an increase in IGF-I levels (Rees *et al.* 1990), but no change in GFR has been demonstrated, either in adults with CRI given rhGH experimentally (Haffner *et al.* 1989) or in children in therapeutic trials of the use of rhGH in short stature of CRI (Tonshoff *et al.* 1992). The effects of rhGH on ERPF in CRI in man have not been reported. More recently, rhIGF-I has been shown to increase both GFR and ERPF in adults with moderate CRI (O'Shea *et al.* 1993). If hyperfiltration is a mechanism for progression of CRF in man, as appears to be the case in small mammals (Brenner *et al.* 1985), then rhGH treatment in children with CRI could potentially lead to a deterioration in renal function in the longterm.

There are other mechanisms whereby rhGH could potentially affect renal function. There are GH and IGF-I receptors on mesangial cells (Arnqvist *et al.* 1988; Aron *et al.* 1989), which could result in expansion of the mesangium and fibrosis. Transgenic mice with prolonged exposure to GH (but not to IGF-I) develop glomerulosclerosis and CRF (Doi *et al.* 1988; Quaife *et al.* 1989; Kawaguchi *et al.* 1991). Prolonged

exposure to GH also causes renal enlargement, as seen in acromegaly (Gerschberg *et al.* 1957) and in transgenic mice secreting high levels of GH (Doi *et al.* 1991). Hypopituitary mice undergoing unilateral nephrectomy have reduced hypertrophy of the remaining kidney (Astarabadi *et al.* 1953).

Many patients with renal transplants have a subnormal GFR, so as with CRI, there are potential concerns that rhGH treatment might result in a deterioration of renal function by causing hyperfiltration. In addition rhGH may affect graft function by other mechanisms. Growth hormone affects the immune system (Bozzola *et al.* 1991), and might therefore precipitate acute rejection or cause progression of chronic rejection (Broyer *et al.* 1994). Published reports give conflicting results of the effect of rhGH on renal graft function (Rees *et al.* 1990; Johansson *et al.* 1990; Fine *et al.* 1992; Bartosh *et al.* 1992; Van Dop *et al.* 1992; Tonshoff *et al.* 1993; Benfield *et al.* 1993; Janssen *et al.* 1993; Hokken-Koelega *et al.* 1994d). It is important to make sure that renal function is not compromised at the expense of improved growth, not least because graft function itself has been shown to have an effect on growth (Tejani *et al.* 1993).

Study Aims

The aim of the study was to determine the immediate (hours), short term (1 week) and the longer term (6 months - 2 years) effects of rhGH on renal function in children with CRI and in those with renal transplants. In order to do this, urea, creatinine, GFR, ERPF and proteinuria were measured before starting rhGH treatment, immediately after receiving the first dose of rhGH, again after 1 week, then 6 monthly or yearly. Glomerular filtration rate and ERPF were estimated from the clearances of inulin and para-aminohippuric acid (PAH) respectively. Additionally, in the transplanted group, the effect of rhGH treatment on the volume of the transplanted kidney was studied. Weight and blood pressure were monitored throughout.

Patients

This study was carried out as part of the BAPN trial of rhGH in CRF, as detailed in Chapter 2. Children attending the London paediatric nephrology centres underwent additional renal function studies and make up this cohort of patients. Details of the 18 children with moderately severe CRI managed conservatively and 16 with renal transplants are given in Table 7.1.

All of the patients in the CRI group had all had a GFR of less than 50 ml/in/1.73m² for more than 1 year. The transplanted patients had received their grafts more than 1 year previously and had had stable graft function for the 6 months before the trial. Mean time from transplantation was 6.5 (2.0 - 11.8) years. Two grafts were from living related donors; 12 were first grafts, 3 were second grafts, and one was a third transplant.

Nine children received triple immunosuppression consisting of azathioprine (60 mg/m²/day), cyclosporin A (at a dose sufficient to maintain plasma levels between 50 and 150 ng/ml), and prednisolone. Seven children were immunosuppressed with azathioprine and prednisolone alone. Prednisolone was given at a mean dose of 9.8 (5.2 - 20.1) mg/ m² on alternate days in 15 children; one child received 7.6 mg/ m² daily.

Retrospective growth data and creatinine values were available for all children for the year preceding entry into the trial.

Study Protocol

Children were seen on day 1 (before treatment), day 8 (after 1 week of rhGH), then 3 monthly up to 1 year. On each occasion blood was sampled in the fasting state for estimation of urea, creatinine, GH and IGF-I. With the exception of day 1, these samples were drawn approximately 12 hours after the preceding dose of rhGH. Height was measured using a wall-mounted stadiometer by the same observer. Blood

pressure was measured in the sitting position. An early morning urine sample was obtained for measurement of microalbumin. Three day dietary assessments were made 6 monthly (2 children received overnight nasogastric feeds).

Methods

Glomerular filtration rate and ERPF were estimated by clearance of inulin (Inutest 25%, Laevosan-Gesellschaft GmbH, Linz/Donau) and para-aminohippuric acid (PAH) (Aminohippurate Sodium 20%, Merck, Sharp and Dohme, West Point, USA) respectively using a standard clearance with urine collection technique.

The procedure was carried out after an overnight fast. A cannula was inserted in each arm, a fasting blood sample withdrawn, then a bolus of 50mg/kg inulin and 5 mg/kg PAH in 50 mls of normal saline given over 3-4 minutes. This was followed by infusion of a maintenance solution of inulin and PAH in 0.45% saline, calculated so as to maintain a plasma inulin level of approximately 250 mg/l ($\text{mg inulin per minute} = 250/1000 \times \text{GFR}$) and 25 mg/l PAH ($\text{mg PAH per minute} = \text{GFR} / 0.2 \times 25/1000$). The maintenance solution was infused at 100 mls/hour, by constant rate infusion pump (IMED Volumetric Pump 960, IMED, Albington, Oxon).

Diuresis was induced by encouraging the child to drink water, 20 ml/kg for the first hour, then 10 ml/kg/hour thereafter. As the study lasted for several hours the child was allowed to eat small non-protein, non-fructose containing snacks. After one hour, steady state was assumed and the bladder emptied. Eight forty minute urine collections (collections nos. 1-8) were made with the child being encouraged to completely empty the bladder on each occasion. A sample of blood was drawn, from the second cannula, at the midpoint of each collection for inulin, PAH and GH. Insulin-like growth factor-I was measured during collections 3 and 8. Growth hormone (0.14 iu/kg) was given by subcutaneous injection on completion of the third collection.

For the children attending the Royal Free Hospital (RFH) and the Hospital for Sick Children, assays were performed at RFH. Inulin was measured spectrophotometrically using a resorcinol method (Roe *et al.* 1949), PAH was determined by the Bratton Marshall reaction (Smith *et al.* 1954), creatinine by an enzymatic method using an RA1000 Autoanalyser (Technicon Instruments Co Ltd., Basingstoke, UK) and IGF-I by radioimmunoassay after acid-ethanol extraction. It is recognised that this method of extraction does not completely remove IGF binding proteins (Powell *et al.* 1986), which can then interfere in the assay. The extent of interference appears to depend on the particular antibody being used. In our assay, acid chromatography, when compared to acid-ethanol extraction, gave comparable results in CRI ($r = 0.979$, $p < 0.0001$) with acid-ethanol extraction giving slightly lower results (acid-ethanol extraction = $[0.906 \times \text{acid chromatography}] - 0.049$) (See also Chapter 8). Growth hormone was measured by immunoradiometric assay (Chapter 8). Urinary microalbumin was measured by an immunoturbidimetric method (Technicon method, Bayer Diagnostics, UK Ltd). Urinary creatinine was measured enzymatically on the same urine samples, and the results expressed as the albumin / creatinine ratio.

For the children attending Guy's Hospital, assays were performed at Guy's Hospital. Inulin was measured enzymatically (Dalton *et al.* 1987), PAH was determined by the Bratton Marshall reaction (Smith *et al.* 1954), and creatinine by an enzymatic method using an RA1000 Autoanalyser (Technicon Instruments Co Ltd., Basingstoke, UK).

Urine samples were analysed for microalbumin by a two-site ("sandwich") enzyme-linked immunosorbent assay (Thomlinson *et al.* 1994). Polyclonal rabbit anti-human albumin immunoglobulin was used both as the capture antibody and, when conjugated to horseradish peroxidase, as signal antibody (Dako, High Wycombe, UK). Intra-assay and inter-assay CV were 4.2% and 11.9% at 10mg/l respectively. The reference range of 0 - 10 mg/mmol creatinine was obtained from normal children (Thomlinson *et al.* 1994).

Urinary N-acetyl glucosaminidase was measured using an automated spectrophotometric method with p-nitrophenyl-B-D-glucosaminide as substrate (Sigma, Poole, Dorset, UK) (Tucker *et al.* 1975). Intra-assay and inter-assay coefficients of variation (CV) were 2.5% and 5.8% respectively at 3.9 mmol/p-nitrophenol/h/mmol creatinine. The reference range of 0 - 100 mmol/p-nitrophenol/h/mmol creatinine was obtained from normal children (Thomlinson *et al.* 1994).

CRI Group

These children were studied for 1 year. Inulin clearances were carried out on day 1, day 8 and at 1 year; on days 1 and 8 rhGH was given at midday rather than in the evening. On day 1, eight urine collections were made; the first 3 to establish baseline clearance. Immediately the third collection was complete, the first subcutaneous injection of rhGH was given and the urine collections continued without interruption. On day 7 rhGH was given in the evening as usual, then exactly the same procedure as above was carried out the following morning (day 8), before and after the eighth injection of rhGH. Baseline clearances (Pre) were taken as the average of the first 3 collections and compared with the average of the last 3 collections (nos. 6-8) (Post) to look for an immediate effect of rhGH. At 1 year, four forty minute urine collections were carried out, 12 hours after the last injection of rhGH. Baseline clearances on day 1, day 8 and after a year were compared, to look at the short and long term effects of rhGH. Four of the youngest children had baseline clearance tests only on each of the 3 days; four forty minute urine collections before rhGH on day 1, and at least 12 hours after the previous dose on day 8 and at 1 year.

Transplanted Group

Inulin and PAH clearances were performed as for the CRI group, however only 4 x 40 minute collections were made on each day. These children were enrolled later than the CRI group, and it had become clear from the CRI group that there was no

immediate effect of rhGH on GFR. The collections were performed before rhGH was given on day 1, and 12 hours after rhGH on the other days. Clearance studies were performed on day 1, 1 week after starting rhGH, then 6 monthly for 2 years. As this arm of the trial was a controlled study, 7 of children were randomised to no treatment in the first year, with all children receiving rhGH treatment in the second year. In the control year clearance studies were performed six monthly, then immediately before starting rhGH, after 1 week, and 6 monthly.

Kidney volume was measured yearly using either an ATL Mark IV or Acuson 128 ultrasound machine, with 3.5 or 5 MHz sector probes. When the whole length of the kidney could not be included in the image, a stand off gel block was used. Repeated measurements of renal length were made until a maximum was reached. Multiple depth and width measurements were taken and averaged. Kidney volume (ml) was estimated using the formula for an ellipse (Dinkel *et al.* 1985).

Rejection episodes were diagnosed by the clinician in charge, and were assumed to have occurred when there was an increase in baseline creatinine of more than 10% on 2 occasions, in the absence of a high cyclosporin A level ($>150\text{ng/ml}$; therapeutic range 50 - 150ng/ml), dehydration, infection or obstruction. Treatment consisted of a 3 day course of oral prednisolone (3mg/kg/day). A renal biopsy was performed if creatinine did not return to baseline after this treatment.

Statistical Analysis

Within each group, statistical analysis was performed using a paired Student's *t*-test, and confirmed using a Wilcoxon matched pairs signed rank sum test. Two-way ANOVA was used to compare repeated measurements in the same group of patients. Correlation was performed by Pearsons Correlation and multiple regression analysis. Within the transplanted group, the treatment and control groups were compared using an unpaired Student's *t*-test.

Results

CRI Group

Fifteen of the 18 children completed one year of treatment. Three children were withdrawn from the trial; two required dialysis after 9 months of treatment and the other received a pre-emptive transplant after 6 months. Data from these children, whilst they were in the trial, have been included and their HV on treatment annualised. There were no other serious adverse events. Height velocity increased from 4.5 (1.7-6.5) cm/yr in the year before treatment to 9.5 (4.8-12.7) whilst on treatment ($p < 0.0001$).

Fourteen children had clearance studies before and after a subcutaneous injection of rhGH on days 1 and 8. Eighteen had clearance studies on both days 1 and 8, and 15 had studies on days 1, 8 and after 1 year.

GH and IGF-I

Growth hormone increased approximately 10-fold in the 3-4 hours following the first subcutaneous injection on both day 1 (Figure 7.1a) and day 8 (Figure 7.1b). Mean fasting GH increased after 1 week of treatment (Table 7.2), but did not accumulate further (Table 7.3). IGF-I did not change in the 3-4 hours after injection on either day 1 or day 8 (Table 7.2), but fasting IGF-I was elevated after 1 week and remained so for the duration of treatment (Table 7.3). Growth hormone and IGF-I results are discussed further in Chapter 8.

GFR

There was no change in GFR in the 3-4 hours after rhGH injection on either day 1 or day 8 (Table 7.2). Eighteen patients had baseline clearances before and after one week of rhGH. There was no change in mean GFR after 1 week of treatment (Table 7.4). There was also no difference after 1 year in the 15 patients who completed the year of

study (Table 7.4). Individual values are shown in Figure 7.2. When a value of zero was substituted for the children who required dialysis, GFR was 16.3 (0 - 59) at 1 year, n=18. This was not significantly different from day 1, $p = 0.20$.

ERPF

Mean ERPF, as determined by clearance of PAH, was unchanged in the 3-4 hours following a subcutaneous injection of rhGH, but was significantly increased after 1 week. Individual values are shown in Figure 7.3. Four patients showed an increase in GFR in addition to an increase in ERPF. This subgroup was not distinguished by baseline renal function, aetiology of renal disease, basal IGF-I or increase in IGF-I after one week.

Mean ERPF remained elevated after a year when compared to day 1 but this was not statistically significant. In the group as a whole, there was a downward trend in filtration fraction, but individually there was much variation and the decrease was not significant.

There was no correlation between baseline GFR, ERPF and the change in GFR and ERPF at 1 week or after 1 year. There was no correlation between absolute IGF-I or IGF-I corrected for age and sex, and GFR or ERPF on day 1, day 8 or at 1 year, nor was there a relationship between the change in either GFR or ERPF and the increase in IGF-I or IGF-I SDS.

Urea and Creatinine

Plasma urea decreased significantly after one week, but returned to pre-treatment values by 3 months. Creatinine was unchanged after 1 week, however there was a significant increase in creatinine after 1 year. Results are shown in Table 7.3

Blood Pressure

Mean systolic and diastolic blood pressures were unchanged by 1 year of rhGH treatment (Table 7.3).

Urinary Albumin Excretion

Albuminuria, expressed as an albumin(mg/l) / creatinine(mmol/l) ratio, was unchanged during the study (0.08 (0.08) on day1; 0.05 (0.05) on day 8; and 0.10 (0.12) at 1 year) in the 15 children with congenital structural problems. The 3 children with FSGS had marked proteinuria.

Weight and Dietary Assessments

Weight was unchanged after 1 week (22.2 (13.3 - 37.0) kg on day 1; 22.3 (13.4 - 36.8) on day 8), but increased to 25.7 (15.5 - 39.2) kg after 1 year of treatment ($p < 0.0001$). Dietary intake of protein did not change during the study period: 2.1 (1.2 - 2.5) gm/kg body weight protein on day 1; 1.9 (1.4 - 2.9) at 6 months; and 1.8 (1.4 - 2.1) at 1 year. Energy intake (kcal/kg body weight) was not affected by treatment: 36 (18 - 59) on day 1; 33 (27 - 47) at 6 months; and 36 (31 - 41) at one year.

Transplanted Group

Fifteen children completed 1 year, 7 completed 18 months and 6 completed 2 years of rhGH treatment. Patient 6 developed fasting hyperglycaemia associated with a raised HBA1c after 9 months, at which time rhGH treatment was stopped.

Two children (patients 7 and 11) had poor graft function at the start of the trial; both had biopsy-proven chronic rejection. Patient 11 received a second graft after 18 months of treatment with rhGH; patient 7 completed 2 years of rhGH but required haemodialysis six months after completion of the trial. Patient 13 had pyelonephritis after 18 months of rhGH and a permanent deterioration of graft function during this

episode. Patient 16 had hypertension and hyponatraemia after the oral water diuresis during the inulin clearance study on day 1; subsequent measurement of renal function was by single injection inulin clearance only.

Height velocity increased significantly in the group receiving rhGH, compared to the children in the control group. Results are given first for all 22 children during rhGH treatment; the treatment and control groups in the first year of study are also compared.

GH and IGF-I

Serum GH and IGF-I levels increased after one week of rhGH and remained elevated thereafter (Table 7.5). There was no relationship between baseline IGF-I or increase in IGF-I and the changes in renal function in individual patients.

GFR

Glomerular filtration rate was significantly increased after one week of rhGH; 52 (28) on day 1 and 57 (29) on day 8 ($p = 0.004$), Table 7.6. Glomerular filtration rate remained increased at six months [62 (31), $p = 0.013$], but not at 1 year [55 (33)], Figure 7.4. Individual values are shown in Figure 7.5. By ANOVA there was a significant increase in GFR with time ($p = 0.036$). There was no further significant change in GFR in those children treated for 2 years: 53 (17 - 65) ml/min/1.73m² at 18 mths ($n=7$); 38 (11 - 51) at 2 years ($n=5$). Despite an increase in GFR, there was an increase in plasma creatinine concentration from 110 (43 - 221) $\mu\text{mol/l}$ to 139 (64 - 317) after one year of treatment, $p = 0.028$ (Table 7.5), presumably because of an increase in muscle bulk. Blood urea decreased after one week of rhGH, but returned to pretreatment values by 3 months (Table 7.5).

ERPF

There was no significant change in ERPF, as measured by clearance of PAH, or filtration fraction during the year of rhGH treatment, Table 7.6. There was no change in ERPF in those children who received rhGH for 2 years: 285 (54 - 598) at 18 mths (n=7); 219 (41 - 469) at 2 years (n=5). Individual patient values are shown in Figure 7.6.

Kidney Volumes

Kidney volume was measured in 10 patients before and during rhGH treatment. Mean volume was 128 (57 - 221) ml on day1 and 133 (57 - 227) after one year (p=0.14). The change in kidney volume was no different from that seen in 5 children studied during the year of no treatment; 114 (76 - 148) on day 1 and 123 (99 - 150) after one year (p=0.38).

Blood Pressure

There was no significant change in systolic or diastolic BP during rhGH treatment (Table 7.5).

Urinary Protein Excretion

There was no significant change in albumin excretion during therapy: day 1 mean (SE) 17.3 (11.6) mg/mmol creatinine; 13.8 (6.7) at 6 months; 13.1 (6.1) at 1 year. There was also no change in the pattern of urinary N-acetyl glucosaminidase excretion: day1 106 (27) mmol/p-nitrophenol/h/mmol creatinine; 120 (20) at 6 months; 123 (26) at 1 year. Four patients had elevated levels on day 1 which persisted with treatment.

Dietary Assessments

There was no significant change in mean (SE) dietary protein intake: 1.3 (0.2) g protein N/kg on day1; 1.1 (0.3) at 6 mths; 1.3 (0.1) at one year. Energy intake was likewise unchanged: 38 (5.3) kcal/ kg on day 1; 32 (5.8) after 6 mths; 42 (7.7) at one year.

Control Data

GFR, ERPF, creatinine and urea did not change in the 7 children studied during their year of no treatment (Table 7.7). Blood pressure, protein excretion and kidney volume were also unchanged. Height velocity was 4.5 (2.8 - 7.8) cm/yr in the year before the trial; 4.1 (1.4 - 5.6) cm/yr in the year of no treatment; and 8.5 (5.3 - 11.1) cm/yr in these 7 children during the first year on rhGH ($p < 0.004$ vs year of no treatment). Comparison of GFR in the treatment and control groups is shown in Figure 7.7.

Discussion

Chronic Renal Insufficiency

Prolonged exposure to consistently high GH levels, such as are found in acromegaly and diabetes, results in an elevation of ERPF and GFR (Ikkos *et al.* 1956; Christiansen *et al.* 1981). Whilst this might appear be beneficial in CRI, if hyperfiltration is indeed a mechanism for progression of CRI in man (Brenner *et al.* 1985), then rhGH treatment in CRI may have a deleterious effect on renal function in the longer term. Patients with acromegaly do not go on to develop chronic renal failure (in the absence of diabetes and hypertension) (Gershberg *et al.* 1957), however rodents with prolonged exposure to very high levels of GH develop glomerular sclerosis (Doi *et al.* 1988; Quaife *et al.* 1989; Kawaguchi *et al.* 1991).

There are a number of mechanisms whereby rhGH could affect renal function. First, as discussed, GH through the action of IGF-I, increases GFR and ERPF in the normal kidney; micropuncture studies in the rat demonstrate that this is due to an effect on glomerular haemodynamics (Hirschberg *et al.* 1991). Second, altered renal function could be related to hypertrophy of the kidney; prolonged exposure to GH causes renal enlargement, as seen in acromegaly (Gershberg *et al.* 1957) and in transgenic mice secreting high levels of GH (Doi *et al.* 1988). Hypopituitary mice undergoing unilateral nephrectomy have reduced hypertrophy of the remaining kidney (Astarabadi *et al.* 1953). Third, transgenic mice with prolonged exposure to GH (but not to IGF-I) develop glomerulosclerosis and chronic renal failure (Doi *et al.* 1988). Finally, rhGH may have an effect on the immune system, and in renal transplantation could precipitate acute rejection or cause progression of chronic rejection (Bozzola *et al.* 1991).

In our CRI patients there was no change in GFR during rhGH treatment, confirming a study in 7 adults with CRI, none of whom showed any change in inulin clearance 24 hours after 3 days of rhGH (Haffner *et al.* 1989). It also supports the findings of the rhGH trials in children with CRI, where GFR, as measured by single injection inulin clearance, was unchanged after 6 months and 1 year (Tonshoff *et al.* 1992) and where the change in calculated creatinine clearance after 24 months was no different on rhGH as compared to placebo (Fine *et al.* 1994).

Two children reached end-stage disease during treatment and required dialysis. It is difficult to disentangle the long term effects of rhGH treatment on renal function from the natural progression of CRI, and it is not known if rhGH treatment affected their clinical course. Both children had FSGS and a rising creatinine before starting treatment. One child showed a 50% increase in ERPF after one week of rhGH; the other showed little change in ERPF. Four children reached end-stage disease in the year following the trial, so perhaps it was surprising that there was not a decline in mean GFR during the study.

Plasma creatinine rose significantly despite a consistent GFR, and presumably reflects an increase in muscle bulk. Fractional excretion of creatinine was unchanged by rhGH treatment (results not shown), therefore the increase in plasma creatinine was not due to a reduction in tubular secretion of creatinine. From our data it is clear that serum creatinine alone is an inadequate marker of GFR in children with CRI during rhGH treatment.

None of the previously mentioned studies have looked at the effects of rhGH on ERPF in CRI in man. After one week of treatment we demonstrated an increase in mean ERPF, to a degree similar to that seen in individuals with normal renal function (approximately 25%) (Hirschberg *et al.* 1989). Three day dietary assessments confirmed that increased ERPF was not due to an increase in protein intake during the trial. There was no change in systemic blood pressure, suggesting the increase in ERPF represents a decrease in renal vascular resistance.

Four children had an increase in GFR as well as ERPF. It was not possible to predict the renal response to rhGH from baseline characteristics and in the group as a whole there was no relationship between the increase in circulating IGF-I and the changes in GFR and ERPF. Autocrine and paracrine actions of IGF-I within the kidney may be as or even more important than circulating IGF-I in mediating the renal effects of rhGH (D'Ercole *et al.* 1984).

These results support a study of four adults with moderate CRI, all of whom showed an increase in GFR and ERPF in response to subcutaneous twice daily injections of rhIGF-I for 5 days (O'Shea *et al.* 1993). Glomerular filtration rate increased between 40-70% with a larger increase in ERPF, such that filtration fraction decreased in each of the four patients. The degree of renal insufficiency was less than in our patients. Interestingly in two of these patients clearance of PAH remained elevated 10-12 days after stopping rhIGF-I, whilst GFR had returned to baseline.

The same group have since undertaken further placebo-controlled studies of IGF-I therapy in patients with severe CRI (6-15 ml/min/1.73m²) (Vijayan *et al.* 1999; Hammerman *et al.* 1999). At the end of 31 days patients treated with IGF-I had a GFR that was 145% of baseline; patients receiving placebo had no change in GFR. Whilst the increase in GFR would seem most probably to be due to an effect of IGF-I on glomerular haemodynamics, 14 days after treatment was stopped GFR remained elevated at 125% of baseline in the treatment group. At this time circulating IGF-I levels had returned to normal, therefore such changes may represent IGF-I induced renal hypertrophy (Hammerman *et al.* 1999).

Micropuncture work in normal rats infused with rIGF-I revealed an increase in GFR and ERPF due predominantly to an increase in efferent arteriolar dilatation and an increase in the ultrafiltration coefficient (Hirschberg *et al.* 1991). The relevance of these changes in CRI (Miller *et al.* 1990; Allen *et al.* 1992) and their application to man, are not clear, but it is interesting to speculate that changes within the glomerulus in CRI, dependent on the severity and aetiology of renal disease, may determine the response, if any, to rhGH or rhIGF-I. Alternatively, as a reduction in renal mass stimulates IGF-I production during compensatory hypertrophy in rodents (Fagin *et al.* 1987; Lajara *et al.* 1989), there may already be increased IGF-I expression in the kidney in CRI which results in a different response to exogenous rGH or rIGF-I. Renal hypertrophy per se may be important in the response to prolonged rGH exposure.

In small mammals, prolonged administration of GH to rats with renal impairment results in glomerular hypertrophy and glomerular sclerosis (Allen *et al.* 1992). Interestingly prolonged exposure to IGF-I causes hypertrophy but not sclerosis of the glomerulus in transgenic mice with normal renal function (Quaife *et al.* 1989). Micropuncture work has shown that glomerular capillary pressure is not raised in response to IGF-I in the normal rat (Hirschberg *et al.* 1991), so perhaps the sclerosis is due to an action of GH which is not mediated via IGF-I-induced haemodynamic changes (Yang *et al.* 1993). As mentioned before patients with acromegaly do not

develop renal disease, despite exposure to high levels of GH. The renal response to GH in man would appear to be different from that in rodents; indeed the rodent reduced renal mass model may not be representative of CRI in man.

Whilst the evidence to date suggests that the renal effects of GH are likely to be due to an effect of IGF-I on glomerular haemodynamics, it is not inconceivable that GH increases secretion of PAH by the proximal tubule. While this cannot be discounted from our data, neither can it be the whole explanation.

Measurement of GFR

It is obviously important in a study such as this that GFR measurements are as accurate as possible. Measurement of GFR by standard inulin clearance with urine collections is the gold standard, but in children it can be a difficult test to perform. It is not ethical to use bladder catheterisation, yet without it one cannot be sure that the child has completely emptied their bladder. Ensuring a high urine flow rate with the use of an oral water diuresis makes it easier to empty the bladder, and so reduces the risk of error. None of our children had overt bladder dysfunction.

To verify the adequacy of bladder emptying, we also calculated the clearance of inulin by the constant infusion without urine collection method; at steady state the amount of inulin infused equals the amount cleared in the urine (Berger *et al.* 1948; Rose *et al.* 1969). There was very close correlation between these methods in our patients (Maxwell *et al.* 1994), confirming that bladder emptying was virtually complete. On 33 occasions GFR was measured by both the urine collection and constant infusion techniques. Glomerular filtration rate, as measured by urine collection, was 20.3 (6.4 - 58.1) ml/min/1.73m² and by constant infusion 21.1 (6.6 - 55.9). The mean difference between the methods was 0.8 (0.5) and the correlation between them was $r = 0.970$, $p < 0.0001$.

Tubular function

As far back as 1962 Corvilian reported reduced urinary phosphate excretion following an injection of hGH. There are GH and IGF-I receptors on proximal tubular cells (Hammerman *et al.* 1986; Rogers *et al.* 1989). Treatment with rhGH could also have an effect on tubular function, as well as glomerular function. As a marker of tubular function we measured urinary excretion of N-acetyl glucosaminidase, a renal tubular enzyme. This was unchanged during rhGH treatment, as was the excretion of albumin in the urine.

Transplanted Patients

The effects of rhGH on renal function are perhaps more pertinent to its use post transplantation. An improvement in height at the expense of graft function is not acceptable. In the group of patients studied, GFR increased after one week and six months of rhGH treatment, and remained elevated, but not significantly so after 1 year. There was no significant change in ERPF or in kidney volume.

The effect of rhGH on GFR after one week is likely to be due to an alteration in glomerular haemodynamics. It is unlikely that any of the other mechanisms outlined above would be acting after only one week of treatment. After six months of rhGH, increased GFR could still be due to altered haemodynamics, or to renal hypertrophy as a result of treatment. With time, however, other influences, such as progression of chronic rejection, have to be taken into account. As in CRI, haemodynamic changes in the transplanted kidney could adversely effect graft function. Despite the increase in GFR, there was also an increase in serum creatinine, presumably reflecting increased muscle bulk.

Our results suggest that the transplanted kidney can respond to rhGH in a similar fashion to the normal kidney, but it seems likely that other factors such as chronic allograft nephropathy intervene to prevent a sustained increase in GFR. It is therefore

difficult to predict the longterm effect of rhGH in renal transplantation from a study of this length. Continued monitoring is required.

An analysis of published studies of rhGH in renal transplantation reported a variable effect on renal function (Chavers *et al.* 1995). However few studies have formally measured GFR in patients with renal transplants during rhGH treatment. One reported no significant change in GFR, as measured by single dose inulin clearance (Tonshoff *et al.* 1993), and the other (Hokken-Koelega *et al.* 1994d) reported no significant changes in GFR as measured by inulin clearance. Other studies of rhGH therapy after renal transplantation quote changes in creatinine or changes in the slope of an inverse creatinine (1/creatinine) plot; however, changes in creatinine do not always reflect a change in GFR. Calculated GFR (Schwartz *et al.* 1976) takes some account of this; a multicentre European study (Johansson *et al.* 1990) showed no significant change in calculated GFR after 1 year. Another study (Benfield *et al.* 1993) showed no significant change in creatinine clearance in 19 children after 1 year, another reported a decrease in creatinine clearance in 5 of 9 patients (Van Dop *et al.* 1992), and yet another reported a decrease in calculated GFR in 3 but an increase in the other 2 patients (Bartosh *et al.* 1992).

Interpretation of these studies is hampered both by the lack of formal measurement of GFR, and by the heterogeneity within patient groups. Slowly growing children tend to have poorer renal function. In one study a rise in creatinine of 90 $\mu\text{mol/l}$ was associated with a decrease in height SDS of -0.17 (Tejani *et al.* 1993). Moreover, GFR decreases with the number of rejection episodes and with length of time from transplantation (Berg *et al.* 1992), so changes in GFR during a study like this may be related to factors other than rhGH.

It is even more difficult to determine the effects of rhGH on the incidence of rejection episodes. Transplant dysfunction that appears clinically to be rejection is treated in our centres with a 3 day course of high dose oral prednisolone; we proceed to a biopsy only if there is no response to this treatment. This does not allow assessment of the

true incidence of rejection, but a similar policy was used in both centres before and during the trial. The incidence of transplant dysfunction was no different during rhGH treatment when compared to the year before. From the literature, the use of rhGH has been associated with biopsy-proven acute rejection in children with previously stable graft function at variable times after commencing rhGH (Johansson *et al.* 1990; Tyden *et al.* 1990; Fine *et al.* 1992; Schwartz and Warady 1992), and also with the progression of chronic rejection (Van Dop *et al.* 1992; Benfield *et al.* 1993). In one report, two children with previously diagnosed chronic rejection had an increase in serum creatinine after the use of rhGH. The creatinine level decreased on stopping rhGH, suggesting a functional effect rather than irreversible changes (Jabs *et al.* 1993).

There have been three major controlled trials including this one, of the use of rhGH after renal transplantation. None have reported an increase in rejection episodes, but risk factors for rejection have been suggested. The Dutch multi-centre study suggested that the use of alternate day steroids was a risk factor for rejection (Hokken-Koelega *et al.* 1994d). The French multi-centre study also reported no overall increase in rejection episodes, but reported an increase in the number of rejection episodes in children who had had rejection episodes in the year prior to the trial (Guest *et al.* 1998). The issue of transplant rejection is discussed further in Chapters 6 and 9.

Conclusion

One year of rhGH treatment in patients with CRI resulted in a significant increase in ERPF after one week, with a return toward baseline values after one year. There was no change in mean GFR during treatment. In the transplanted group, GFR increased after one week and six months of treatment, but had returned toward starting values after one year. There was no change in ERPF. In both groups blood pressure and urinary protein excretion was unchanged. Kidney volume was unchanged in the transplanted group.

Table 7.1 Patient details in the CRI and transplant groups

	CRI	Renal Transplant
Number Boys / Girls	15 / 3	12 / 4
Age	9.1 (4.9 - 13.9)	13.1 (9.4 - 16.4)
Prepubertal / Pubertal	17 / 1	11 / 5
Ht SDS	-3.0 (-4.8 to -1.8)	-2.9 (-4.5 to -1.6)
GFR	19 (9 - 58)	52 (18 - 117)
<u>Aetiology of renal Disease</u>		
Congenital Structural	15	9
Congenital Nephrotic Syndrome		1
Cystinosis		1
Glomerulonephritis		1
FSGS*	3	2
HUS**		1
Interstitial Nephritis		1

* focal segmental glomerulosclerosis ** haemolytic uraemic syndrome

Table 7.2 Growth hormone (GH), insulin-like growth factor-I (IGF-I), glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) in the CRI group before and after an injection of rhGH on days 1 and 8

	Day 1		Day 8	
	<i>Pre</i> <i>n=14</i>	<i>Post</i> <i>n=14</i>	<i>Pre</i> <i>n=14</i>	<i>Post</i> <i>n=14</i>
GH (mU/l)	12.2 (0.5 - 35.8)	105.9 (33 - 166.8)	14.0 (1.8 - 34.7)	163.6 (80 - 291)
IGF-I (ng/ml)	145 (51 - 255)	129 (32 - 222)	238 (59 - 546)	236 (89 - 590)
GFR (ml/min/1.73m ²)	22 (9 - 60)	21 (10 - 60)	23 (6 - 58)	25 (7 - 53)
ERPF (ml/min/1.73m ²)	81 (35 - 281)	80 (35 - 247)	99 (42 - 272) *	104 (38 - 272) *

* *p* = 0.03 vs Day 1

IGF-I was measured during collections 3 and 8

GH, GFR and ERPF are the averaged values of collections 1-3 and 6-8

Table 7.3 Renal function, growth hormone (GH), insulin-like growth factor-I (IGF-I) and blood pressure in the CRI group during rhGH treatment

<i>n</i> =18	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year
CREATININE (umol/l)	274(66 - 472)	273 (69 - 486)	306 (57 - 562)*	328 (76 - 572)*	382 (78 - 889)*	375 (85 - 574)*
UREA (mmol/l)	18.2 (9.5 - 30.0)	13.7 (5.3 - 22.5)**	17.0 (6.5 - 26.6)	18.2 (6.9 - 24.1)	19.9 (9.8 - 32.2)	19.2 (7.2 - 30.0)
GH (mU/l/l)	11.0 (0.5 - 37.7)	21.9 (2.4 - 54.9)*	28.2 (2.4 - 63.3)*	21.1 (2.7 - 58.3)	22.0 (2.2 - 60.6)	24.4 (4.0 - 76.3)***
IGF-I (ng/ml)	148 (46 - 315)	302 (83 - 673)*	305 (111 - 584)*	349 (119 - 745)*	327 (87 - 699)*	362 (101 - 786)*
<u>BP (mmHg)</u>						
SYSTOLIC	97 (90 - 110)	96 (87 - 109)	96 (85 - 112)	98 (75 - 112)	98 (85 - 115)	98 (75 - 120)
DIASTOLIC	58 (40 - 70)	59 (45 - 70)	60 (50 - 70)	61 (40 - 78)	61 (50 - 80)	64 (45 - 85)

* $p < 0.01$ vs day 1, ** $p = 0.0001$ vs day 1, *** $p < 0.05$ vs Day 1

Fasting GH: normal range < 10 mU/l

IGF-I: normal range is age related

Table 7.4 Renal function in theCRI group in the 18 patients who had clearance studies on both days 1 and 8, and in the 15 children who had clearance studies on day 1 and after 1 year

	1 Week		1 Year	
	Day 1 <i>n</i> = 18	Day 8 <i>n</i> = 18	Day 1 <i>n</i> = 15	1 Year <i>n</i> = 15
Baseline GFR (ml/min/1.73m ²)	19 (9 - 58)	22 (6 - 56)	19 (9 - 58)	20 (9 - 59)
Baseline ERPF (ml/min/1.73m ²)	77 (34 - 271)	96 (33 - 276) *	78 (34 - 271)	99 (24 - 428)
Filtration Fraction	0.26 (0.15 - 0.40)	0.25 (0.11 - 0.41)	0.25 (0.15 - 0.40)	0.24 (0.11 - 0.38)

* *p* = 0.005 vs Day 1

Table 7.5 Renal function, growth hormone (GH), insulin-like growth factor-I (IGF-I) and blood pressure in the transplanted group during rhGH treatment

<i>n</i> =16	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year
CREATININE (umol/l)	110 (43-221)	111 (37-248)	114 (33-235)	114 (37-240)	126 (47-252)	133 (47-317)*
UREA (mmol/l)	12.1 (6.0-26.7)	10 (3.0-22.0)**	10.8 (2.7-22.5)	11.9 (3.0-33.1)	11.6 (3.2-22.2)	12.4 (5.3-29.5)
GH (mU/l)	6.2 (1.1-14)	23.0 (5.0-52.1)**	13.1 (1.5-30.6)	17.6 (3.7-43.6)	12.8 (1.0-43.6)	20.5 (0.7-59.3)
IGF-I (ng/ml)	242 (95-410)	540 (117-1002)**	613 (166-903)	590 (105-891)	649 (89-958)	681 (424-948)
<u>BP (mmHg)</u>						
SYSTOLIC	110 (100-124)	114 (100-128)	111 (95-130)	114 (105-125)	108 (90-125)	115 (100-130)
DIASTOLIC	70 (50-80)	76 (50-95)	71 (51-100)	75 (60-90)	68 (60-80)	69 (60-80)

* $p=0.03$ vs day 1, ** $p<0.001$ vs day 1

Fasting GH: normal range < 10 mU/l

IGF-I: normal range is age related

Table 7.6 Renal function in all transplanted patients during their first year of rhGH treatment

	DAY 1	DAY 8	6 MTHS	1 YEAR
GFR (mls/min/1.73m ²)	52 (18 - 117)	57 (20 - 118)*	62 (18 - 133) **	55 (15 - 109)
ERPF (mls/min/1.73m ²)	237 (82 - 495)	244 (88 - 539)	271 (95 - 572)	254 (56 - 538)
FILTRATION FRACTION	0.23 (0.14 - 0.42)	0.25 (0.14 - 0.48)	0.25 (0.13 - 0.36)	0.24 (0.15 - 0.32)

* $p=0.004$ vs. *day 1*, ** $p=0.013$ vs. *day 1*

Table 7.7 Renal function, growth hormone (GH), insulin-like growth factor-I (IGF-I) and blood pressure in the transplant control group during the first year of study

	Day 1	6 Mths	1 Year
Creatinine (umol/l)	95 (49 - 152)	94 (42 - 146)	93 (37 - 142)
Urea (mmol/l)	8.0 (5.1 - 10.9)	8.2 (5.0 - 11.2)	8.7 (6.1 - 14.0)
GH (mU/l)	3.3 (0.3 - 14.0)	9.3 (0.3 - 48.7)	6.3 (0.8 - 12.4)
IGF-I (ng/ml)	263 (174 - 451)	265 (170 - 370)	238 (178 - 309)
GFR (mls/min/1.73m ²)	65 (34 - 105)	77 (32 - 145)	66 (29 - 117)
ERPF (mls/min/1.73m ²)	238 (115 - 449)	289 (130 - 743)	240 (93 - 495)
Filtration Fraction	0.29 (0.19 - 0.36)	0.28 (0.20 - 0.34)	0.28 (0.17 - 0.42)
Systolic BP	103 (88 - 110)	115 (105 - 128)	113 (105 - 120)
Diastolic BP	63 (58 - 70)	75 (70 - 80)	73 (60 - 80)

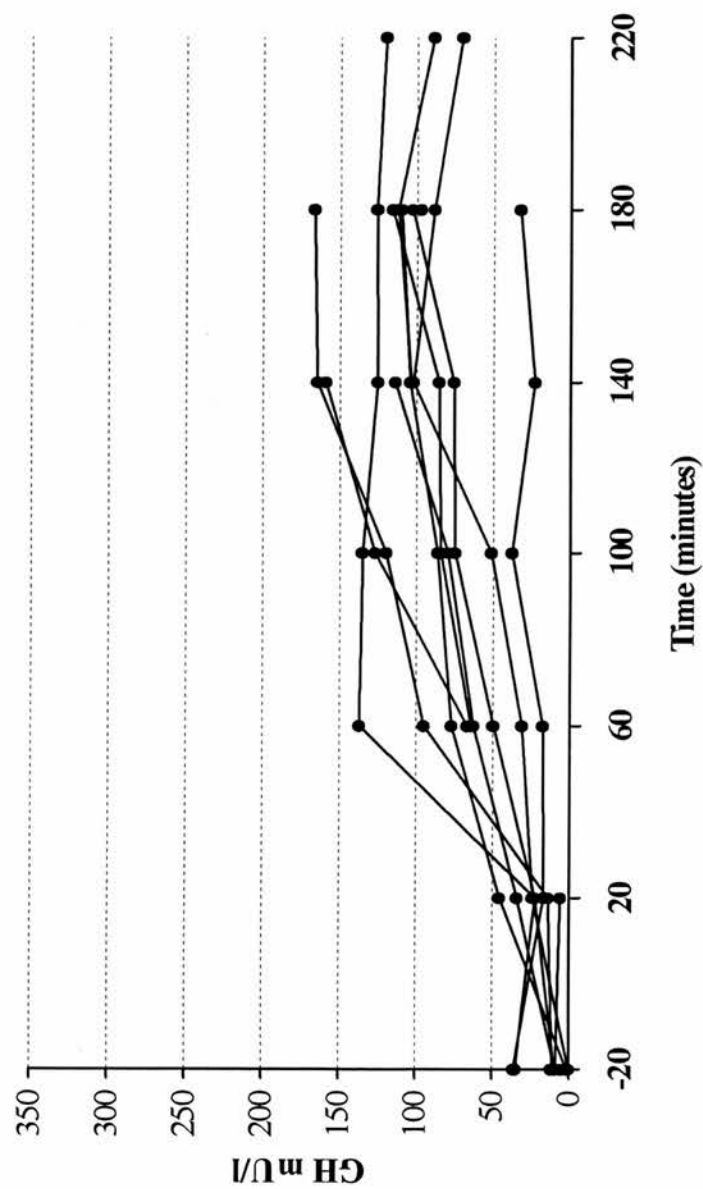


Figure 7.1a Growth hormone (GH) profiles after the first subcutaneous injection of rhGH on day 1 of the study in the CRI group.

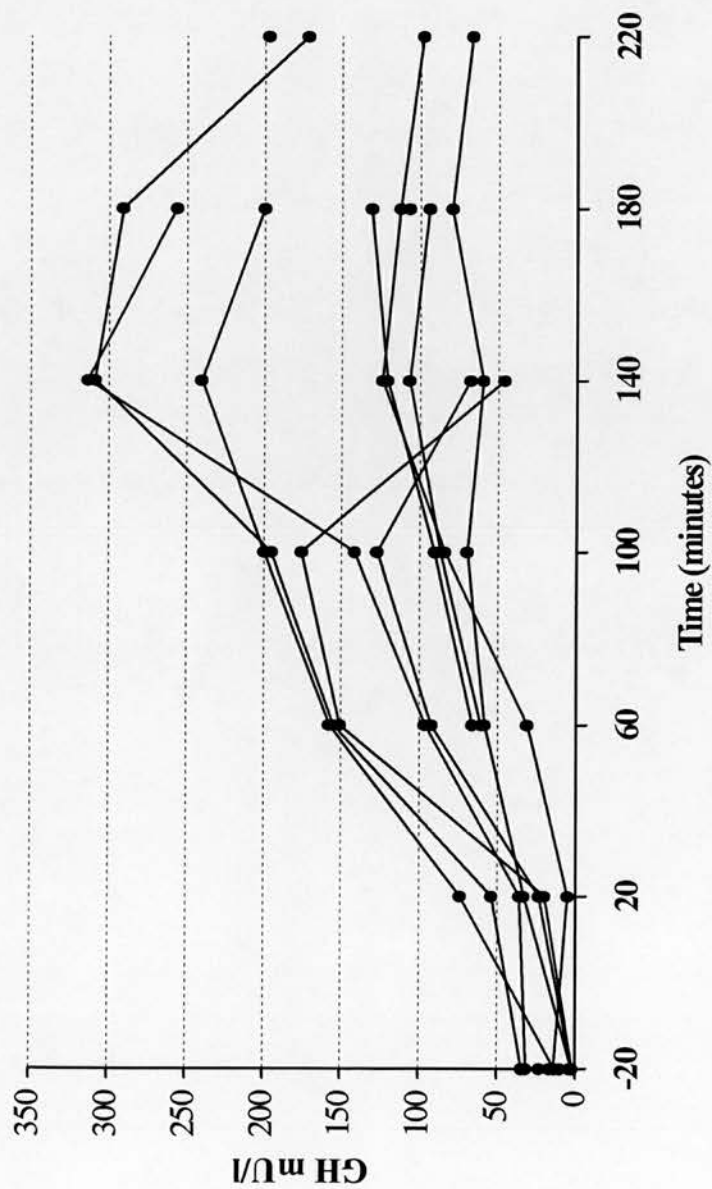


Figure 7.1b Growth hormone (GH) profiles after the subcutaneous injection of rhGH on day 8 of the study in the CRI group.

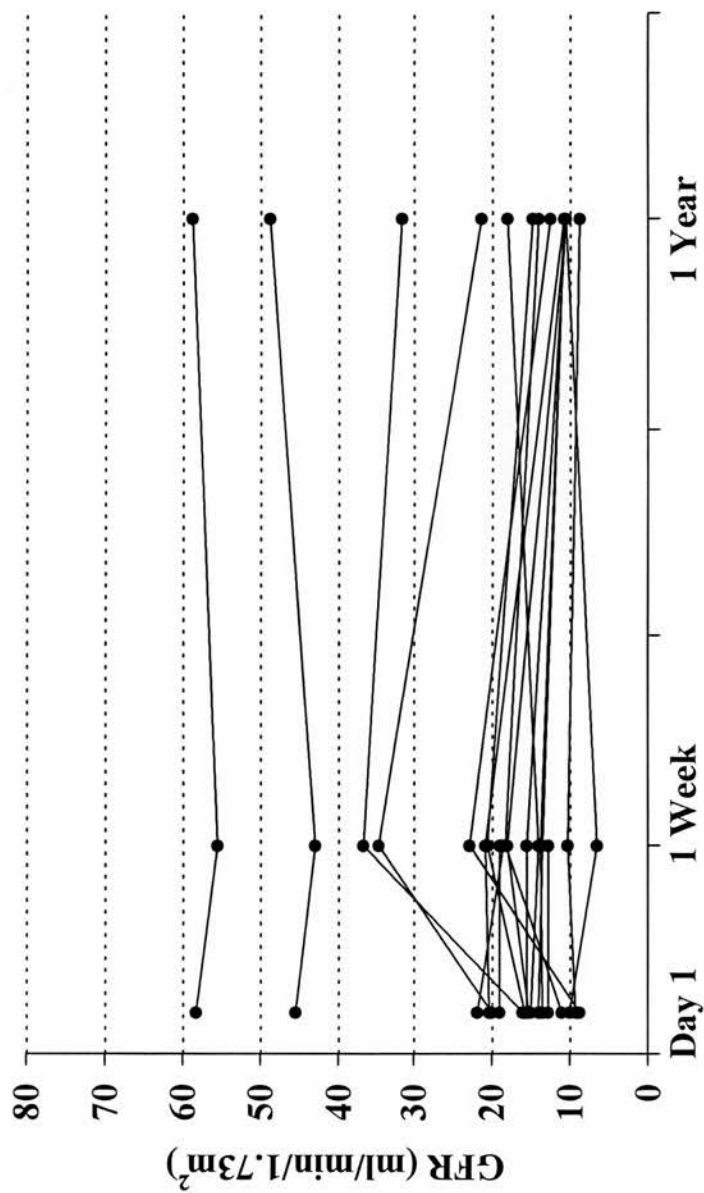
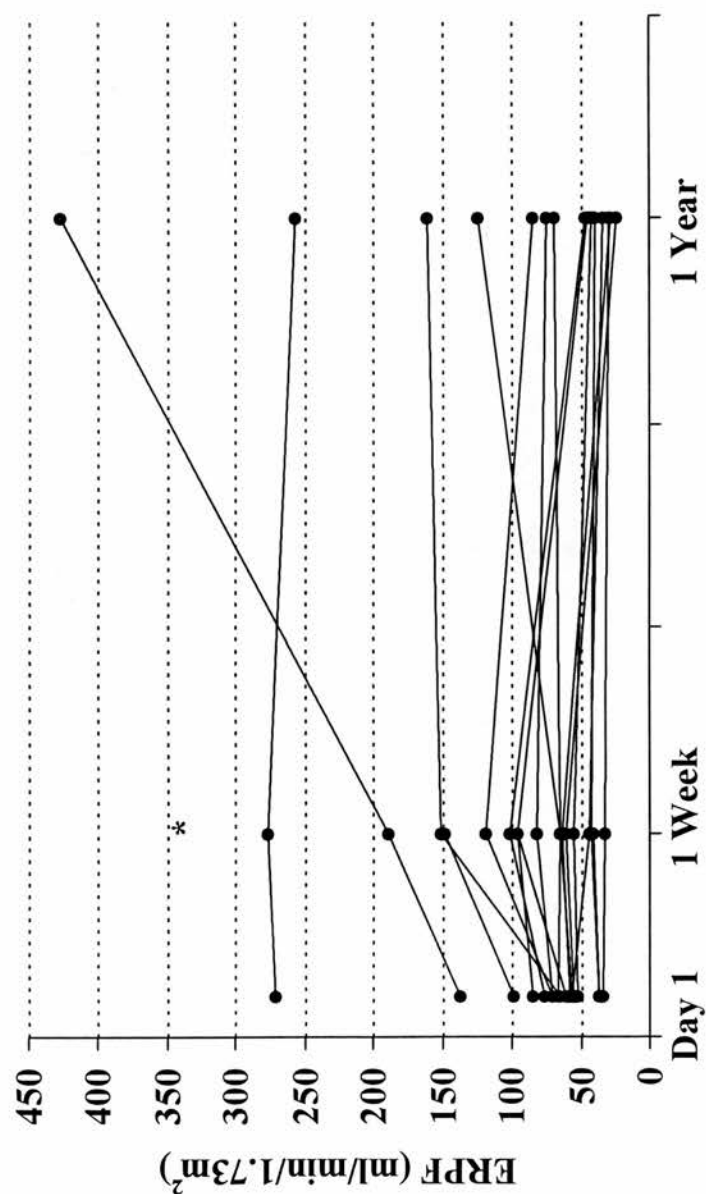


Figure 7.2 Individual GFR measurements in the CRI patients on day 1, after 1 week and 1 year of rhGH treatment. Mean GFR was unchanged during treatment.



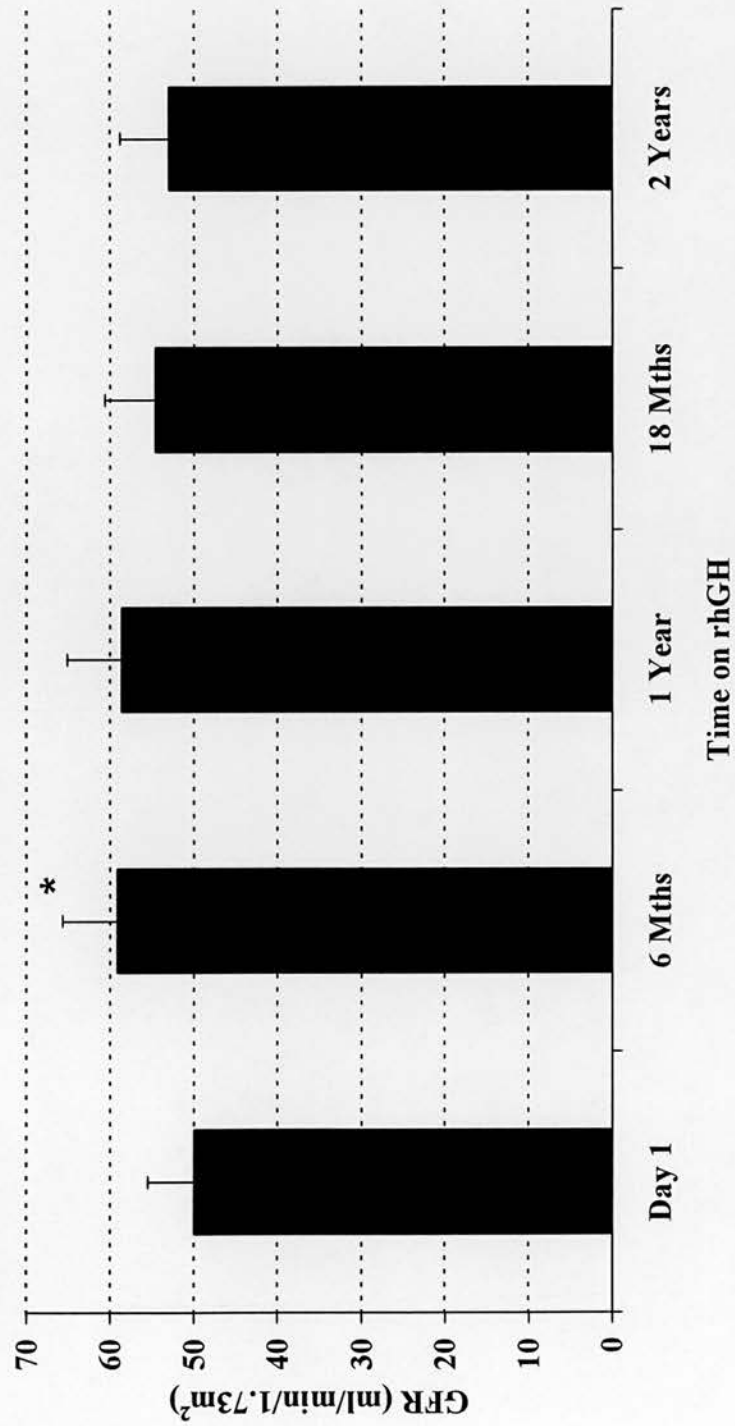


Figure 7.4 GFR (ml/min/1.73m²) in all transplanted patients who received 2 years of rhGH treatment,
 * $p = 0.01$ vs day 1.

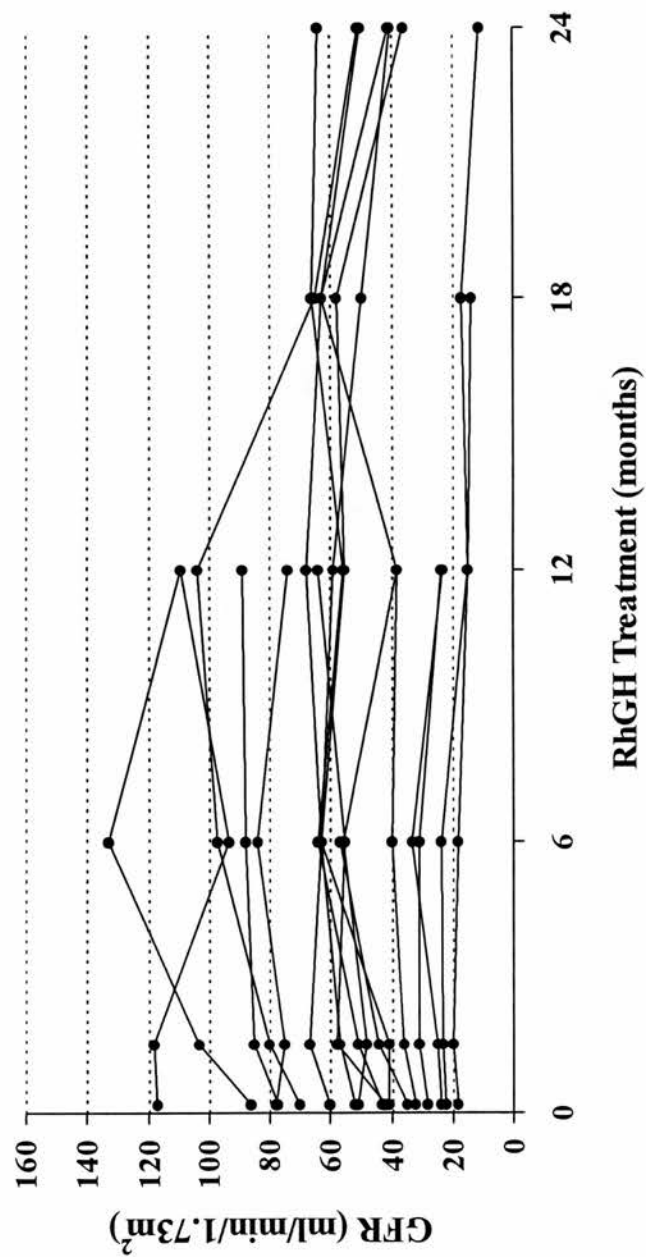


Figure 7.5 GFR (ml/min/1.73m²) in the transplanted group before, after 1 week, and at 6 monthly intervals during rhGH treatment.

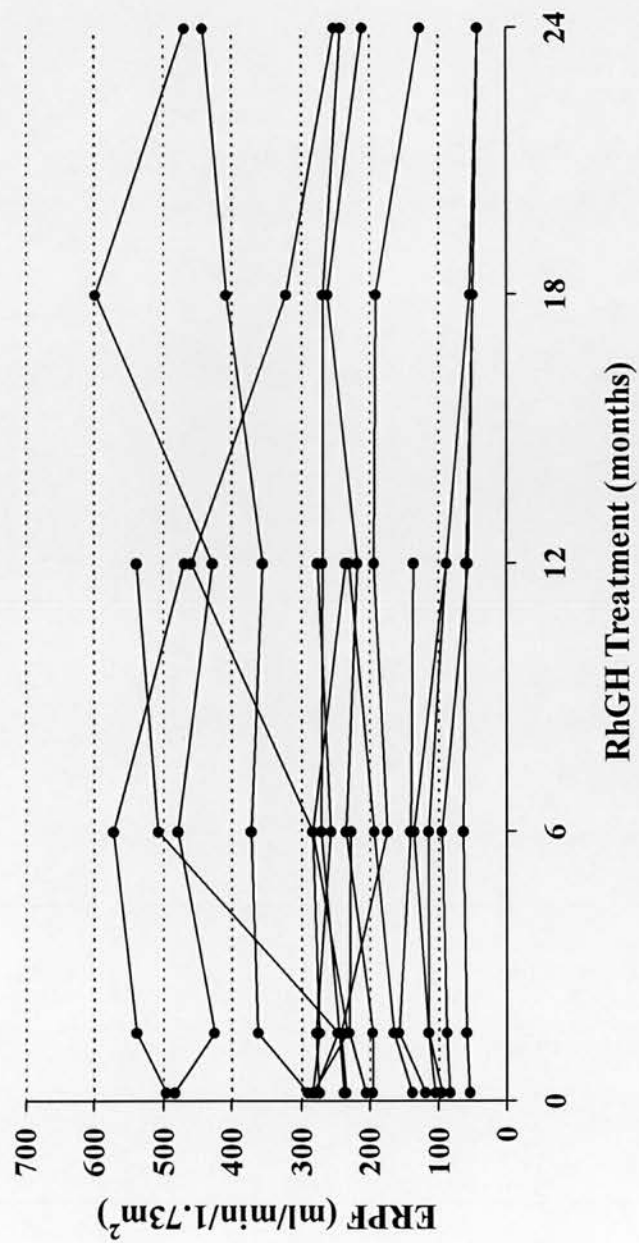


Figure 7.6 Renal plasma flow (ERPF) (ml/min/1.73m²) in the transplanted group before, after 1 week, and at 6 monthly intervals during rhGH treatment.

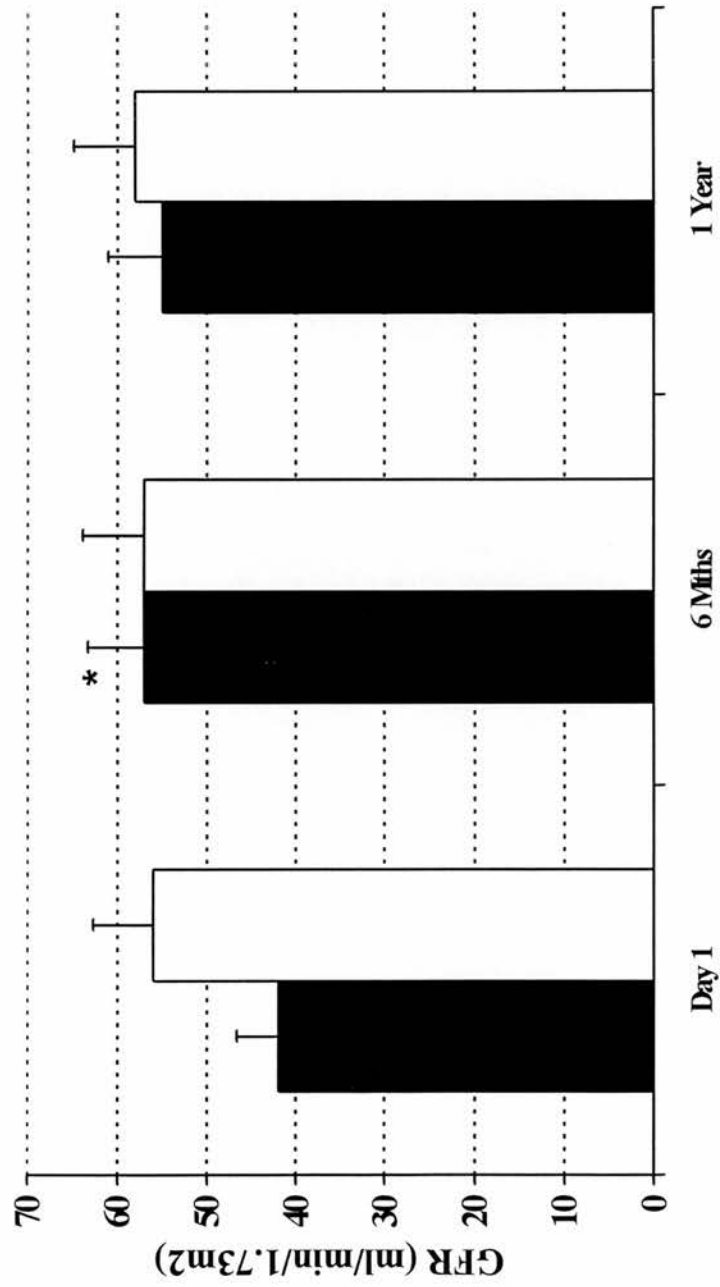


Figure 7.7 GFR (ml/min/1.73m²) in the transplant treatment ■ and control □ groups during the first year of study, * $p = 0.04$ vs. day 1.

Chapter 8: Insulin-Like Growth Factors And Insulin-Like Growth Factor Binding Proteins in Children with Chronic Renal Insufficiency and Following Renal Transplantation Before And After Treatment With Growth Hormone

Introduction

Poor growth in CRF is attributed to a state of GH resistance, where there are high levels of GH, but reduced expression of hepatic GH receptors and low normal levels of its main mediator IGF-I (Blum *et al.* 1991; Chan *et al.* 1993). In addition it has long been recognised that the IGF binding capacity of uraemic serum is high (Powell *et al.* 1987). This has been attributed to raised levels of IGFBPs which have a high affinity for IGFs (Powell *et al.* 1987, 1993; Lee *et al.* 1989; Blum *et al.* 1991). As a result IGF bioactivity of uraemic serum is low (Phillips *et al.* 1984; Baxter *et al.* 1986; Blum *et al.* 1991).

To date, six IGFBPs have been described (Ballard *et al.* 1989; Rosenfeld *et al.* 1994). Quantitatively IGFBP-3 is the most important binding protein and acts as a reservoir for IGF-I in the circulation (Baxter RC *et al.* 1991). IGFBP-3 fragments are said to accumulate with increasing renal failure (Blum *et al.* 1991), and then fall after renal transplantation (Tonshoff *et al.* 1996). IGFBP-1 and -2 have also been proposed as possible inhibitors of growth in renal failure (Burch *et al.* 1990; Lee PDK *et al.* 1993; Cox *et al.* 1994; Powell *et al.* 1997a).

Whilst most emphasis has been placed on IGFBP-3 as an inhibitor of growth in renal failure (Mehls *et al.* 1990; Blum *et al.* 1991; Tonshoff *et al.* 1991b), there is evidence to suggest that the role of IGFBP-3 is more likely to be one of growth promotion rather than growth suppression. IGFBP-3 is GH dependent (Wood *et al.* 1988) and *in vitro* can potentiate the action of IGF-I (Blum *et al.* 1989). IGFBP-3 levels increase during successful treatment of short stature with rhGH in renal failure (Rees and Maxwell 1996b).

To address the role of IGFBPs in short stature of renal failure, two groups of poorly growing children were studied; a group with conservatively managed CRI, and a group with renal transplants. Insulin-like growth factors and IGFBPs were measured before and during a year of rhGH therapy.

Western ligand blot analysis (WLB) allowed semi-quantification of IGFBPs -1 to -4. In addition IGFBPs -1, -2 and -3 were measured by radioimmunoassay (RIA) or immunoradiometric assay (IRMA). IGFBP-3 was also measured by western immunoblot (WIB) analysis using an anti-IGFBP-3 antibody. WIB permits separate identification of intact IGFBP-3 and IGFBP-3 fragments; IRMA quantifies total IGFBP-3 (i.e. combined intact and fragments), and WLB detects only intact IGFBP-3. We also measured the ability of serum from the 2 groups of children to break down intact recombinant IGFBP-3 into fragments (Guidice *et al.* 1990; Hossenlopp *et al.* 1990) so called *in vitro* IGFBP-3 proteolysis.

The relationship between IGF and IGFBP measurements and age, age-related height and HV, GFR and steroid dosage before and during the use of rhGH was studied.

Patients

Seventeen children (3 girls) with CRI and 11 (3 girls) with renal transplants being treated at two paediatric nephrology centres in London were studied. Patient details at the start of rhGH treatment are given in Table 8.1. Entry criteria were as outlined in Chapter 2.

In the CRI group, 14 children had congenital structural problems, two had focal segmental glomerulosclerosis and the other developed renal failure following cardiac surgery. The transplanted group had received their grafts 5.7 (1.9 - 11.6) years previously. All were taking azathioprine (60 mg/m²/day) and prednisolone 9.6 (5.2 - 20.1) mg/m² on alternate days. Four were also on cyclosporin A (at a dose sufficient to maintain plasma levels of 50-150 ng/ml). Eight had congenital structural problems,

the remaining 3 children had developed renal failure secondary to cystinosis, haemolytic uraemic syndrome and glomerulonephritis of unknown aetiology. Height velocity in cm/yr was available for the year prior to the study.

Methods

The children were seen on day 1, day 8 and then at 3 monthly intervals for 1 year. Fasting blood samples were drawn on each occasion; before starting rhGH on day 1, and approximately 12 hours after the previous injection of rhGH on subsequent days. The blood was centrifuged, separated and the serum stored at -30°C.

IGF-I

Serum IGF-I was measured after acid ethanol extraction of its binding proteins, by RIA using a polyclonal rabbit antiserum (R557A), raised against highly purified human IGF-I. The level of detection of the assay was 13 mg/l. The inter-assay CV was 9.0%, 6.5% and 4.7% at analyte levels of 45, 243 and 698 mg/l respectively, with an intra-assay CV of 10.5%, 10.1% and 5.1% at 75, 196 and 698 mg/l. We have previously shown acid ethanol extraction to be comparable to acid chromatography for measurement of IGF-I in renal disease (Powell *et al.* 1986; Maxwell *et al.* 1996b). Values are reported as age-related SDS.

IGF-II

Serum IGF-II was measured by a non-competitive time resolving immunofluorimetric assay (Frystyk *et al.* 1995). The intra-assay CV was < 5% and the inter-assay CV was < 10%.

IGFBP-3 (IRMA)

IGFBP-3 was measured using a coated-tube IRMA kit (Diagnostic Systems Laboratories, Webster, Texas, USA). The minimum detection limit was 0.5 mg/l. The inter-assay CV was 4.2% and 3.1% at 6.0 and 21.1 mg/l; the intra-assay CV was 8.1% and 5.4% at 2.2 and 9.8 mg/l respectively. This antibody detects both intact IGFBP-3 and fragments (Powell *et al.* 1993).

The molar ratio of IGF-I and IGF-II (both 7.6 kDa) to IGFBP-3 (40 Da) was estimated. As IGFBP-3 circulates in many forms this can only be an approximation.

Western Ligand Blotting (WLB) and Western Immunoblotting (WIB) of IGFBPs

WLB was performed in all of the children studied. WLB detects IGFBPs 1-4; only the intact form of IGFBP-3 is recognised. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and ligand blot analysis were performed according to the method of Hossenlopp (Hossenlopp *et al.* 1986) as previously described (Flyvbjerg *et al.* 1992). Two ml of serum was subjected to SDS-PAGE (10% polyacrylamide) under non-reducing conditions with unlabelled IGF-I (Bachem, Bubendorf, Switzerland). All samples from each subject were analysed in the same gel.

The identity of the IGFBPs detected on the WLB was confirmed using specific immunoblot analysis for IGFBP-1, IGFBP-2, IGFBP-3 and IGFBP-4 (UBI, Lake Placid, New York, USA) with ³⁵S-protein A (specific activity 500 Ci/mmol) (Amersham International, Amersham, Bucks, UK). IGFBP-3 appears as an intact 38-42 kDa doublet, a 30 kDa fragment and a smaller fragment of 16 kDa. Sera from 6 children in each group were subjected to IGFBP-3 WIB at each time point in the study. These children were selected at random by a blinded investigator. Total IGFBP-3, and each of the fragments were analysed. Pooled control serum from 10 age-matched children (8 boys, 2 girls) was run on the same gel.

IGFBP-3 Protease Activity

To assess the ability of CRI and transplant sera to break down intact IGFBP-3, IGFBP-3 protease assays were run in 5 children selected at random by a blinded investigator, and were performed as previously described (Lamson *et al.* 1991). Recombinant ^{125}I -IGFBP-3 (30000 cpm) (Diagnostic Systems Laboratories, Webster, Texas, USA) was incubated for 18 hours at 37°C with 2 ml of serum samples from each patient then subjected to SDS-PAGE as described above. Electrophoresed gels were fixed in a solution of 7% acetic acid, dried and autoradiographed. The degree of proteolysis of each sample was given as the percentage of the optical density per lane which appeared in the proteolytic cleavage product bands. Pooled control (as above) and term pregnancy sera were run on each gel.

Quantification of WLB, WIB and IGFBP-3 Protease Activity

Autoradiographs were quantified by densitometry using a Shimadzu C9001 PC dual wavelength flying spot scanner. The relative density of the bands was measured as arbitrary absorbance units per square millimetre (AU/mm^2).

IGFBP-2 (RIA)

IGFBP-2 was measured by RIA (Diagnostic Systems Laboratories, Webster, Texas, USA). The intra-assay and interassay CV were < 5% and < 10% respectively.

IGFBP-1(RIA)

IGFBP-1 was measured by a specific RIA. Purified antigen was obtained from Dr SLS Drop (Rotterdam, Holland). Tracer was prepared by iodination using the chloramine T method followed by separation on a Sephadex G75 column. Bound and free fractions were separated with a solid phase second antibody, donkey anti-rabbit coated cellulose (SAC-CEL, from IDS, Boldon, Tyne and Wear, UK). Minimum

detection limit of the assay was 6 mg/l. The inter-assay CV at 55 mg/l was 6%, and the intra-assay CV at 35 mg/l was 4%.

Insulin

Insulin was measured using an immunoassay (Enzymun-Test Insulin, Boehringer Mannheim GmbH Diagnostica, FRG). The reference range for fasting subjects is 3-17 mU/l. Inter-assay CV was 9.2% and 4.9% at 19.7 and 92.1 mU/l respectively. Intra-assay CV was 8.6%, 8.1% and 3.6% at 7.1, 11.4 and 33.0 mU/l.

Statistical Analysis

Comparison of measurements within each group at multiple time points was carried out by ANOVA. Comparison of results at two time points was performed by a Student's *t*-test. Correlation was performed using Pearson's correlation coefficients and backward multiple regression analysis. Statistical significance was assumed when a *p* value of 0.05 or less was obtained.

In the CRI group HV was negatively correlated with age and therefore HVSDS values were used in the correlations with IGFs and IGFBPs. In the slightly older transplanted group, the timing of puberty makes the interpretation of HVSDS difficult, and as HV was unrelated to age, HV was used in preference to HVSDS.

Results

Height velocity and height SDS increased significantly in both groups of children during treatment with rhGH (Table 8.1). There was no significant change in GFR during the year of study (Table 8.1).

IGF-I

At the start of treatment mean IGF-I SDS values were similar in both groups and were within the normal range [CRI: -1.06 (-4.16 to 2.47); transplanted group: -1.11 (-3.92 to 2.27)], Figure 8.1. During treatment, IGF-I SDS increased to a mean value of 3.03 (-1.23 to 8.11) in the CRI group, $p < 0.001$ vs. day 1, and 6.65 (0.50 to 17.10) in the transplanted group, $p < 0.001$ vs. day 1. The average increase in IGF-I SDS during treatment was significantly greater in the transplanted compared with the CRI group ($p = 0.008$). Absolute IGF-I values are given in Tables 8.2a and 8.2b.

IGF-II

At the start of the study, IGF-II SDS values were greater in the CRI group than in the transplanted group [CRI: 2.65 (0.50 - 7.26); transplanted group: 1.03 (-2.51 to 3.18), $p = 0.02$], Figure 8.2. IGF-II SDS increased significantly during treatment in the CRI group (averaged value during treatment 4.50 (2.27 - 9.58), ANOVA during treatment $p < 0.0001$), but not in the transplanted group (averaged value during treatment 1.00 (-1.73 to 4.23), ANOVA during treatment $p = 0.31$), Figure 8.2. The increase in IGF-II in the CRI group was evident by 1 week ($p < 0.0001$). Absolute values for IGF-II are given in Tables 8.2a and 8.2b.

IGFBP-3

At the start of treatment IGFBP-3 SDS, when measured by IRMA, was within the normal range in both groups of children [CRI: 0.44 (-0.53 to 2.00) and transplanted group: 0.28 (-1.06 to 1.38)], Figure 8.3. IGFBP-3 SDS increased significantly during rhGH treatment, with averaged values of 2.93 (1.53 to 5.57) in the CRI group and 2.12 (0.17 to 4.07) in the transplanted group during the study, $p < 0.001$ vs. day 1 for both groups, Figure 8.3. Absolute values of IGFBP-3 by IRMA and the molar ratio of IGFs to IGFBP-3 are shown in Tables 8.2a and 8.2b. During rhGH treatment, the ratio IGFs/IGFBP-3 decreased in the CRI group ($p = 0.004$), despite improved growth. The ratio did not change in the transplanted group.

Comparison of IGFBP-3 by IRMA, WLB and WIB

The IRMA assay measures total IGFBP-3 (both intact and IGFBP-3 fragments). Absolute values of IGFBP-3 by IRMA were similar in the two groups on day 1 of the study, Tables 8.2a & 8.2b, Figure 8.4. By WLB analysis however, IGFBP-3 was lower in the transplanted group compared to the CRI group on day 1 (Figure 8.4), and as WLB detects only intact IGFBP-3, this implied that there was less intact and more fragmented IGFBP-3 in the transplanted group.

To investigate this finding further, sera from 6 children in each group were randomly selected by a blinded investigator and subjected to WIB using an IGFBP-3 antibody. The WIB results are shown as % values in Table 8.3; the absolute data are given in Figure 8.5. On day 1, total IGFBP-3 when measured by WIB was similar in both groups (CRI: 49.3 (18.6) AU/mm²; transplanted: 54.5 (18.2), $p = 0.64$), so confirming the IRMA findings, and in fact was no different to pooled control serum (59.9 (22)) run on the same gels. Confirming the discrepancy in day 1 results for WLB and IRMA, the % IGFBP-3 present as the intact form was less in the transplanted group compared to the CRI group, and to pooled control serum. There was no difference between the CRI group and the control sera ($p = 0.17$), Table 8.3.

On day 1, the % of IGFBP-3 present as the 30 kDa fragment was higher in the transplanted group compared to both the CRI group and to the control serum, Table 8.3. There was no difference between the CRI and control sera ($p = 0.41$). In the transplanted group, the % of 16 kDa fragment was not increased compared to the CRI group ($p = 0.52$), but the % was increased in both the transplanted and CRI groups compared to the control serum. In short, the children with renal transplants had normal total IGFBP-3, but the % present as intact IGFBP-3 was low. The children with CRI had a small increase in 16 kDa fragments, but normal amounts of intact IGFBP-3.

The pattern of intact and fragmented IGFBP-3 in individual patients can also be expressed as % "*in vivo* proteolysis", which is the ratio of both 30 and 16 kDa

IGFBP-3 fragments to total IGFBP-3. On day 1, mean *in vivo* proteolysis in the transplanted group was increased compared to both the CRI group and to the control serum, Table 8.3. In the transplanted group *in vivo* proteolysis before rhGH treatment was unrelated to prednisolone dose.

During rhGH treatment

IGFBP-3 values increased during treatment in both groups by all three methods employed. Comparing the pattern of different forms of IGFBP-3 by WIB, the only significant change during rhGH treatment was of an increase in % intact IGFBP-3 in the transplanted group ($p = 0.04$). Associated with this there was a decrease in *in vivo* proteolysis during rhGH treatment in the transplanted group (Day 1 = 70 (10)%, 1 Year = 60 (9)%, $p = 0.008$). There was no such change in *in vivo* proteolysis in the CRI group. A representative immunoblot from one of the transplanted children during treatment is shown in Figure 8.6.

In vitro IGFBP-3 Protease Activity

Having confirmed a decrease in intact IGFBP-3 in the transplanted group, we went on to measure the ability of both transplant and CRI sera to induce proteolysis of intact recombinant IGFBP-3. Protease activity was not increased above control values at the start of treatment in either of the groups, and was much reduced compared to pooled term pregnancy serum run on the same gels, Figure 8.7. There was no significant change in *in vitro* protease activity in either group during rhGH therapy.

IGFBP-2

On day 1, IGFBP-2 measured by RIA was greater in the CRI than transplanted groups, $p = 0.006$, Tables 8.2a & 8.2b, Figure 8.8. IGFBP-2 did not change significantly during treatment in either group, Tables 8.2a & 8.2b. At the start of the study, IGFBP-2 when measured by both RIA and WLB was significantly correlated with the dose of prednisolone ($r = 0.623$, $p = 0.04$) and ($r = 0.919$, $p < 0.001$)

respectively, Figure 8.9. IGFBP-2 was negatively correlated with GFR ($r = 0.624$, $p = 0.04$) in the transplanted group, Figure 8.10. In the CRI group, IGFBP-2 was also inversely proportional to GFR ($r = -0.876$, $p = 0.05$), Figure 8.10.

IGFBP-1

In the CRI group on day 1, mean IGFBP-1 was elevated at 244 (74.6 - 424) mg/l, (normal range is age related but is < 50 mg/l). IGFBP-1 SDS are shown in Figure 8.11. There was a small but significant decrease in IGFBP-1 levels during the year of rhGH ($p = 0.04$). Fasting insulin values increased slightly after 1 week ($p < 0.01$) then gradually returned to baseline values during the year of treatment, Figure 8.12.

In the transplanted group mean IGFBP-1 SDS was elevated on day 1, but to a lesser degree than in the CRI group, Figure 8.11. In contrast to the CRI group, there was a marked decrease in IGFBP-1 after one week of rhGH ($p = 0.0025$), with the mean value remaining within the normal range thereafter. Fasting insulin levels rose significantly, and to a greater degree than in the CRI group, Figure 8.12. The normal inverse relationship between insulin and IGFBP-1 was preserved in the transplanted group, with the change in one mirroring the other, however in individual patients there was no significant correlation between the change in fasting insulin and fasting IGFBP-1 before treatment ($r = 0.20$), nor between change in IGFBP-1 and change in insulin after one week ($r = 0.13$). The greatest decrease in IGFBP-1 was seen in those children with the highest pre-treatment IGFBP-1 levels ($r = 0.921$, $p < 0.0001$).

There was no correlation between fasting insulin and *in vivo* proteolysis of IGFBP-3 in either group, either before or after rhGH treatment.

IGFBP-4 (WLB)

There was no difference between IGFBP-4 levels in the two groups on day 1: CRI group 13.8 (9.4); transplanted group 9.2 (9.1), $p = 0.21$, Figure 8.13. In both groups

IGFBP-4 was significantly correlated with IGFBP-1: CRI ($r = 0.798$, $p < 0.0001$); transplanted group ($r = 0.703$, $p = 0.001$). During rhGH treatment, there was a trend for IGFBP-4 to increase in the CRI group (ANOVA, $p = 0.06$), but not the transplanted group. The mean value during treatment in the CRI group was 14.7 (9.1); this was no different from the value in the transplanted group 17.1 (11.3), $p = 0.56$.

Correlations

CRI group

Correlations between growth and IGF / IGFBPs in the CRI group, both before and during rhGH treatment, are shown in Table 8.4. Before treatment, height SDS was positively correlated with IGF-II SDS ($r = 0.593$, $p = 0.01$), Figure 8.14, and with IGFBP-3 SDS ($r = 0.674$, $p = 0.005$), Figure 8.15. HVSDS in the year before treatment was greater in those children with the highest IGF-I SDS ($r = 0.560$, $p = 0.01$), Figure 8.16, and was negatively correlated with IGFBP-1 SDS ($r = -0.535$, $p = 0.03$), Figure 8.17. During rhGH the increase in height SDS was negatively correlated with IGF-II SDS during treatment ($r = -0.502$, $p = 0.04$).

Transplanted group

Similar correlations between growth and IGFs and IGFBPs for the transplanted group are shown in Table 8.5. As many of the children were of a pubertal age, calculation of HVSDS can be problematic. Therefore in this group of children HV rather than HVSDS was used for the correlations. Height velocity was shown to be independent of age (Figure 8.18). These growth parameters were not correlated with any of the variables tested, and in fact the results were similar if HVSDS was substituted for HV. The increase in height SDS in the year before treatment was also analysed as this may be more representative of recent growth. In the year before treatment, the increase in height SDS was positively correlated with IGF-I SDS ($r = 0.579$, $p = 0.06$), and with GFR ($r = 0.611$, $p = 0.046$), but not with prednisolone dose

Height velocity during treatment was positively correlated with mean IGF-I SDS during rhGH ($r = 0.692$, $p = 0.02$), Figure 8.19. Increase in height SDS during treatment was positively correlated with IGF-I SDS at the $p = 0.06$ level.

IGFs / IGFBPs and Renal Function

The relationship between growth factors and renal function is shown in Table 8.6. IGFBP-2 was negatively correlated with GFR in both groups. In the transplanted group, IGF-II was also negatively correlated with GFR.

Discussion

These detailed studies of IGFs and IGFBPs in short children with CRI and renal transplants before and during rhGH revealed interesting findings. In CRI previous reports have suggested that IGF binding capacity is increased and IGF bioactivity reduced; IGFBPs are thought to be responsible, with IGFBP-3 proposed as the main binding involved (Blum *et al.* 1991). However using 3 different assays, IGFBP-3 was not elevated in either group, and contrary to previous reports (Blum *et al.* 1991; Powell *et al.* 1993), there was no marked increase in IGFBP-3 fragments in the CRI group. Indeed there were more fragments and less intact IGFBP-3 in the transplanted than CRI children, a previously unreported finding.

Also of note in the transplanted group was a correlation between IGFBP-2 and prednisolone dose, an unexpected finding. Growth retardation is common post transplantation, and is related to several factors including the dose of steroids (Offner *et al.* 1991). In this study, IGFBP-2 when measured by 2 different methods was correlated with the dose of steroid (Figure 8.9), and it is interesting to speculate that IGFBP-2 may be involved in the growth inhibition due to steroids. IGFBP-2 is increased in GH deficiency, insulin deficiency and malnutrition (Hardouin *et al.* 1987). GH secretion is reduced in children with renal transplants on steroids (Schaefer *et al.* 1991) and steroids can induce insulin resistance and catabolism, so perhaps the

relationship is not altogether surprising. No other IGF or IGFBP measured was correlated with the dose of prednisolone.

There are a number of factors pointing to IGFBP-2 as an inhibitor of growth; it is inhibitory in culture systems *in vitro* (Burch *et al.* 1990). IGFBP-2 is elevated in CRI, and height SDS is inversely correlated with IGFBP-2 in CRI (Tonshoff *et al.* 1995a; Powell *et al.* 1997a). It is possible that IGFBP-2 is merely a marker for relative GH deficiency, although IGFBP-2 was higher in CRI than the transplanted group, and there was no reduction in IGFBP-2 in either group during rhGH treatment. The relationship between IGFBP-2 and prednisolone dose is in contrast to the results of short-term administration of dexamethasone to healthy volunteers, where a reduction in IGFBP-2 was seen (Miell *et al.* 1993).

There is more reason to suspect IGFBP-2 is related to growth suppression than IGFBP-3. In children with CRI and in those on dialysis both IGFBP-2 SDS and IGFBP-3 SDS are increased, however IGFBP-2 levels are relatively greater than IGFBP-3 in both groups of children (Tonshoff *et al.* 1995a, 1996). In experimental uraemia, increased gene expression of IGFBP-1 and -2 have been reported (Tonshoff *et al.* 1995b). IGFBP-3 is GH dependent and indeed in both groups of patients was positively related to indices of growth. IGFBP-2 was the only IGFBP which was negatively correlated with GFR in both groups in this study.

In CRI, IGFBP-3 has variously been reported to be elevated (Lee *et al.* 1989; Blum *et al.* 1991; Powell *et al.* 1993; Tonshoff *et al.* 1995a) or normal (Baxter *et al.* 1986; Hodson *et al.* 1992; Lee D-Y *et al.* 1993; Rees and Maxwell 1996b). IGFBP-3 is higher in children on dialysis than in those with residual native renal function (Tonshoff *et al.* 1996), so that differences in residual GFR, and different assays and methodology may explain this discrepancy. However, the lack of a consistent finding of elevated IGFBP-3 in children with CRI on conservative management suggests that IGFBP-3 is not an important factor in short stature of CRI. IGFBP-3 fragments were slightly elevated compared to control sera, but were less so than in the transplanted

children, making them an unlikely source of growth suppression. IGFBP-3 is GH dependent and the significant increase in IGFBP-3 during treatment also go against IGFBP-3 being a major inhibitor of growth in CRI. IGFBP-3 levels rose by approximately 50% during rhGH treatment.

IGFBP-3 is a 38-42 kDa glycoprotein which has the ability to bind to an acid-labile subunit (ALS) to form a high molecular weight complex (150 kDa) which tends to keep IGF-I in the circulation (Baxter RC *et al.* 1991). Fragments of IGFBP-3 have been described, and although these fragments can bind IGF-I, they do so with less affinity than intact IGFBP-3 (Baxter *et al.* 1986; Gargosky *et al.* 1992). Proteolysis of IGFBP-3 was first demonstrated in the serum of pregnant women and is presumed to be a mechanism to increase the availability of IGF-I to the foetus (Guidice *et al.* 1990; Hossenlopp *et al.* 1990). Proteolysis can be described in two different ways; *in vitro* and *in vivo* proteolysis. The former describes the ability of serum to break down intact (recombinant) IGFBP-3 into fragments, and the latter describes the pattern of fragments present in serum (% of total IGFBP-3 present as fragments).

IGFBP-3 proteolysis has also been reported in malnutrition, after major surgery and in GH resistant and deficient states (Davies *et al.* 1991; Holly *et al.* 1993), and would appear to be a response to a catabolic state. Glucocorticoids are catabolic, whereas rhGH is anabolic, and in keeping with this *in vivo* proteolysis was increased in the transplanted group before treatment, and there was a significant reduction in *in vivo* proteolysis during rhGH. IGFBP-3 *in vivo* proteolysis is increased in untreated diabetics, and has been shown to decrease following insulin treatment (Bereket *et al.* 1995). Whilst we did not demonstrate a correlation between *in vivo* proteolysis and insulin levels before rhGH treatment in our patients, there was a reduction in *in vivo* proteolysis and an increase in insulin following rhGH treatment.

Reduced intact IGFBP-3 might lead to a reduction in the half-life of circulating IGF-I. Alternatively an increase in IGFBP-3 fragments, as was found in the transplanted group, might interfere with the interaction between IGF-I and its receptor, assuming

the fragments are able to leave the circulation and enter the extra-cellular space. Either of these mechanisms might contribute to growth retardation in transplanted children and this may be a mechanism of steroid induced growth retardation in CRF (Unterman and Phillips 1985), however there was no obvious relationship between *in vivo* proteolysis and prednisolone dose, although the numbers are small.

Neither the serum from the transplanted group nor the CRI group was able to breakdown intact recombinant IGFBP-3 (no *in vitro* proteolysis). Presumably in the transplanted group the IGFBP-3 is being broken down elsewhere.

The main determinant of IGFBP-1 is insulin status; levels of insulin and IGFBP-1 are inversely correlated (Lee PDK *et al.* 1993). Insulin promotes growth, and levels rise during rhGH treatment. In this study, the increase in insulin was greater in the transplanted than CRI group, and there was a greater decrease in IGFBP-1 in the transplanted group. One explanation for this may be the degree of insulin resistance in uraemia (Mak and DeFronzo 1992), which could obliterate the normal inverse relationship between insulin and IGFBP-1. Either resistance to the actions of insulin or an increase in IGFBP-1 may be related to growth suppression in CRI. IGFBP-1 can be inhibitory *in vitro* (Ritvos *et al.* 1988; Burch *et al.* 1990). Steroids also can result in insulin resistance (Fennell *et al.* 1993), but the normal relationship between insulin and IGFBP-1 still exists in the transplanted patients.

A correlation was found between IGFBP-4 and IGFBP-1 in both groups, the significance of which is unclear. IGFBP-4 has been found to be inhibitory in culture systems (Conover *et al.* 1993).

Since the work for this thesis was completed, specific antisera to IGFBPs 4-6 have subsequently become available. IGFBP-6 levels are raised in CRF, in proportion to the degree of renal failure (Powell *et al.* 1997b). IGFBP-6 levels were found not to be related to indices of growth in CRF, to bind IGF-II rather than IGF-I, and to be

unaffected by rhGH treatment (Powell *et al.* 1997b). IGFBP-6 may therefore modulate IGF-II in CRF, but its role in short stature is unclear.

Serum IGFBP-4 is also elevated in CRF but levels are not related to height SDS. Treatment with rhGH increased IGFBP-4 by 26% (Powell *et al.* 1999). IGFBP-5 is not raised in CRF, but levels double with rhGH treatment (Powell *et al.* 1999). IGFBP-5 was found to correlate with IGF-I, IGF-II and with growth rates in CRF, and has been postulated to be involved in the growth response to rhGH (Powell *et al.* 1999).

Whatever the role of IGFBPs in short stature of renal failure, it is clear that rhGH treatment results in a substantial increase in IGF-I in both groups and an increase in IGF-II in the CRI group. Both IGF-I and IGF-II are able to stimulate linear growth in hypophysectomized rats (Schoenle *et al.* 1985). Powell recently reported an increase in free IGF-I in children with CRF receiving rhGH treatment (Powell *et al.* 1997a).

Comparison of the two groups of patients studied gives useful information. Despite the same dose of rhGH, the increase in IGF-I (adjusted for age) was lower in the CRI than in the transplanted group. The findings in CRI are consistent with growth hormone resistance. Growth hormone levels are high, but the mediator, IGF-I is not elevated (Powell *et al.* 1986; Blum *et al.* 1991). In experimental uraemia, the number of GH receptors on hepatic cells is reduced (Chan *et al.* 1993; Tonshoff *et al.* 1994), as is expression of IGF-I mRNA (Tonshoff *et al.* 1995b). There were significant correlations between indices of growth and age-related IGFs in the CRI group before treatment. Absolute IGF-I was in the low normal range in our group of CRI patients and rose 2 fold with rhGH treatment. The dose of rhGH in CRI is twice the replacement dose used in GHD.

With the same dose of rhGH, the increase in IGF-I SDS in the transplanted group was significantly greater. Growth hormone secretion is reduced in transplanted patients receiving steroids (Schaefer *et al.* 1991), and the greater increase in IGF-I would fit

with GH 'replacement' rather than overcoming GH resistance. The response to treatment was positively correlated with IGF-I SDS. Interestingly the growth response to treatment was similar in both groups.

IGFBP-3 values before treatment, and the increase in IGFBP-3 in response to treatment, was similar in both groups. This would fit with IGFBP-3 being dependent on GH rather than IGF-I (Vaccarello *et al.* 1993). IGF-II was within normal limits in the transplanted group at baseline, but was elevated in the CRI group and rose further during rhGH treatment. This discrepancy is difficult to explain, but adds to the impression that the GH / IGF-I axis is less disturbed in the transplanted than CRI group. In normal children, both IGF-I and IGFBP-3 correlate with indices of growth (Blum *et al.* 1993); this relationship is retained in transplanted children but not in those with CRI.

Conclusion

Levels of IGFBP-3 in poorly growing children with CRI and renal transplants were not elevated. There was a reduction in intact IGFBP-3 and an increase in *in vivo* proteolysis in the transplanted group. IGFBP-2 was increased in the CRI group, and in the transplanted group was correlated with the dose of prednisolone and GFR. IGFBP-1 was elevated in both groups, particularly the CRI group, in whom the usual inverse relationship with insulin was lost. In CRI IGFBP-1 and IGFBP-2 may be related to poor growth, whereas in transplanted children poor growth may be related to IGFBP-2 and -3.

Table 8.1 Patient details in the CRI and transplanted groups

	CRI Group	Transplant Group
Boys/Girls	14/3	8/3
<u>At the start of Treatment</u>		
AGE (years)	8.7 (4.1 - 13.9)	12.9 (10.0 - 16.5)
Prepubertal / Pubertal	16 / 1	8 / 3
GFR (mls/min/1.73m ²)	20 (9 - 58)	51 (22 - 117)
Height SDS	-2.8 (-4.0 to -1.8)	-3.0 (-3.7 to -1.7)
HV before rhGH (cm/yr)	4.8 (1.7 - 6.6)	4.0 (1.4 - 5.6)
<u>After 1 year of rhGH</u>		
GFR (mls/min/1.73m ²)	21 (9 - 59)	57 (15 - 113)
Height SDS	-2.0 (-3.6 to -1.1) *	-2.4 (-3.6 to -1.3) *
HV during rhGH (cm/yr)	10.0 (5.9 - 12.7) *	8.8 (3.0 - 12.5) *

Mean (range)

* $p < 0.001$ vs previous year

Table 8.2a: IGFs and IGFBPs during rhGH treatment in the CRI group

	Day 1	Day 7	3 Months	6 Months	9 Months	1 Year
IGF -I (mg/l)	148 (82)	302 (179)	304 (162)	348 (189)	327 (212)	362 (217)*
IGF-II (mg/l)	1094 (230)	1287 (246)	1235 (269)	1315 (245)	1353 (237)	1355 (230)*
IGFBP-1 (mg/l) (RIA)	244 (106)	229 (98)	248 (128)	241 (99)	212 (106)	192 106)**
IGFBP-2 (mg/l) (RIA)	1.25 (0.28)	1.27 (0.42)	1.19 (0.32)	1.24 (0.29)	1.30 (0.22)	1.33 (0.33)
IGFBP-3 (mg/l) (IRMA)	3.75 (0.71)	5.19 (1.37)	5.50 (1.19)	6.02 (1.27)	5.98 (1.35)	6.47 (1.79)*
IGFs/IGFBPs	1.96	1.81	1.65	1.62	1.65	1.56 ^g
Insulin (mU/l)	9.7 (6.4)	14.0 (9.0) ^f	12.7 (6.7)	10.5 (4.2)	11.3 (4.3)	10.6 (3.2)

* Increase in values over the year by ANOVA, $p < 0.001$; ** Decrease in values over the year by ANOVA, $p = 0.04$; ^f Decrease in values over the year by ANOVA, $p = 0.004$; ^g $p < 0.01$ vs day 1

Table 8.2b: IGFs and IGFBPs during rhGH treatment in the transplanted group

	Day 1	Day 7	3 Months	6 Months	9 Months	1 Year
IGF -I (mg/l)	255 (64)	537 (174)	620 (200)	636 (138)	693 (229)	696 (168) *
IGF-II (mg/l)	1032 (237)	1021 (272)	1051 (204)	1087 (275)	981 (265)	988 (223)
IGFBP-1 (mg/l) (RIA)	91 (71)	31 (23)	59 (41)	46 (21)	44 (27)	55 (34) **
IGFBP-2 (mg/l) (RIA)	0.66 (0.35)	0.66 (0.39)	0.61 (0.33)	0.58 (0.30)	0.53 (0.25)	0.46 (0.26)
IGFBP-3 (mg/l) (IRMA)	4.00 (0.96)	5.19 (1.5)	5.90 (1.44)	5.44 (1.53)	5.63 (1.71)	5.52 (1.33) *
IGFs/IGFBPs	1.83	1.75	1.73	1.94	1.81	1.65
Insulin (mU/l)	14.7 (12.1)	27.8 (12.8) [†]	22.8 (17.9)	25.4 (15.2)	25.5 (16.5)	21.1 (20.5)

* Increase in values over the year by ANOVA, $p < 0.001$; ** Decrease in values over the year by ANOVA, $p < 0.001$; [†] $p = 0.03$ vs day 1.

Table 8.3: IGFBP-3 western immunoblot (WIB) of CRI and renal transplant serum; baseline results before starting rhGH treatment

<u>IGFBP-3 WIB</u>	CRI	Renal Transplantation	Controls
Intact %	46.0 (31.3 - 69.6)	29.9 (15.0 - 42.8) * ^a	55.2 (39.7 - 64.2)
30 kDa Fragment %	47.6 (28.4 - 62.9)	62.3 (48.3 - 72.3) ** ^b	42.3 (33.2 - 59.1)
16 kDa Fragment %	6.4 (2.0 - 11.7)	7.8 (3.0 - 12.7) ***	2.5 (0 - 4.9) ^a
<i>In vivo proteolysis %</i>	54.0 (30.4 - 68.7)	70.1 (57.2 - 85.0) * ^a	43.3 (34.1 - 59.8)

Results are shown as mean (range) and are the % of the total IGFBP-3 present as intact, 30 kDa and 16 kDa fragments. * $p < 0.001$ vs controls, ** $p = 0.004$ vs control, *** $p = 0.007$ vs control, ^a $p = 0.03$ vs CRI group, ^b $p = 0.04$ vs CRI.

Table 8.4 Correlations in the CRI group before and during rhGH treatment

PRE-TREATMENT

<u>Baseline Height SDS</u>		<u>r</u>	<u>p</u>
	IGF-I SDS	0.049	0.85
	IGF-II SDS	0.593	0.012
	IGFBP-1 SDS	0.100	0.70
	IGFBP-2 SDS	-0.767	0.13
	IGFBP-3 SDS	0.683	0.04
<u>Baseline HVSDS</u>			
	IGF-I SDS	0.551	0.022
	IGF-II SDS	-0.052	0.84
	IGFBP-1 SDS	-0.535	0.027
	IGFBP-2 SDS	0.416	0.49
	IGFBP-3 SDS	0.241	0.53

DURING TREATMENT

<u>Δ Height SDS</u>		<u>r</u>	<u>p</u>
	IGF-I SDS	-0.185	0.48
	IGF-II SDS	-0.502	0.041
	IGFBP-1 SDS	-0.283	0.271
	IGFBP-2 SDS	-0.267	0.66
	IGFBP-3 SDS	-0.381	0.31
<u>HVSDS</u>			
	IGF-I SDS	0.296	0.25
	IGF-II SDS	-0.169	0.52
	IGFBP-1 SDS	-0.335	0.19
	IGFBP-2 SDS	-0.290	0.64
	IGFBP-3 SDS	0.331	0.38

Table 8.5 Correlations in the transplanted group before and during rhGH treatment

PRE-TREATMENT

<u>Baseline Height SDS</u>		<u>r</u>	<u>p</u>
	IGF-I SDS	-0.270	0.42
	IGF-II SDS	-0.351	0.29
	IGFBP-1 SDS	-0.128	0.71
	IGFBP-2 SDS	-0.215	0.52
	IGFBP-3 SDS	0.097	0.78
<u>Baseline HVSDS</u>			
	IGF-I SDS	0.344	0.30
	IGF-II SDS	-0.260	0.44
	IGFBP-1 SDS	-0.061	0.86
	IGFBP-2 SDS	-0.319	0.34
	IGFBP-3 SDS	-0.216	0.52

DURING TREATMENT

<u>Δ Height SDS</u>		<u>r</u>	<u>p</u>
	IGF-I SDS	0.577	0.063
	IGF-II SDS	-0.287	0.39
	IGFBP-1 SDS	-0.018	0.96
	IGFBP-2 SDS	-0.254	0.45
	IGFBP-3 SDS	-0.099	0.77
<u>HVSDS</u>			
	IGF-I SDS	0.325	0.33
	IGF-II SDS	-0.172	0.61
	IGFBP-1 SDS	0.024	0.94
	IGFBP-2 SDS	-0.109	0.75
	IGFBP-3 SDS	-0.131	0.70

Table 8.6 Correlations between IGFs / IGFBPs and renal function before rhGH treatment

<u>CRI group</u>	<u>r</u>	<u>p</u>
IGF-I	-0.023	0.93
IGF-II	0.095	0.72
IGFBP-1	0.170	0.51
IGFBP-2	-0.876	0.055
IGFBP-3	0.106	0.79
<u>Transplanted Group</u>		
IGF-I	-0.375	0.26
IGF-II	-0.628	0.038
IGFBP-1	-0.227	0.50
IGFBP-2	-0.624	0.04
IGFBP-3	-0.263	0.43

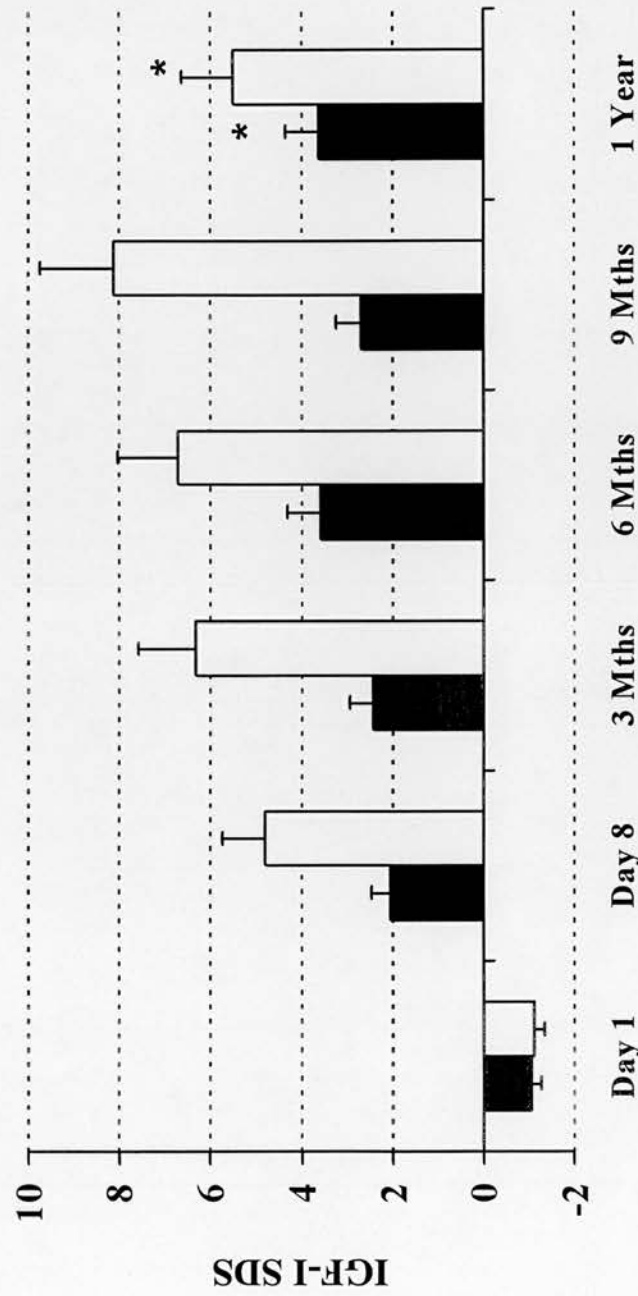


Figure 8.1 IGF-I SDS before and during rhGH treatment in the CRI ■ and transplanted □ groups. IGF-I SDS increased significantly in both groups, * $p < 0.001$ ANOVA. Results are shown as mean and SE.

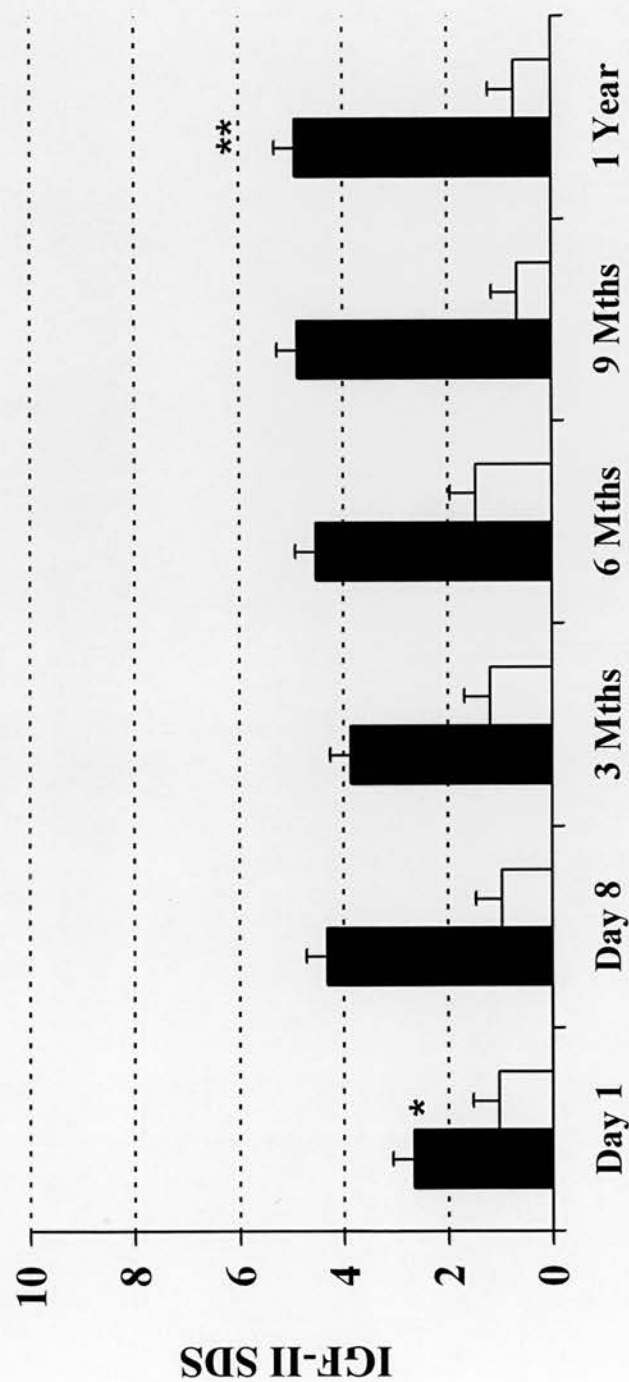


Figure 8.2 IGF-II SDS before and during rhGH treatment in the CRI ■ and transplanted □ groups. Levels were higher in the CRI vs. transplanted group on day 1, * $p = 0.02$. IGF-II SDS increased significantly in the CRI group, ** $p < 0.0001$ ANOVA Results are shown as the mean and SE.

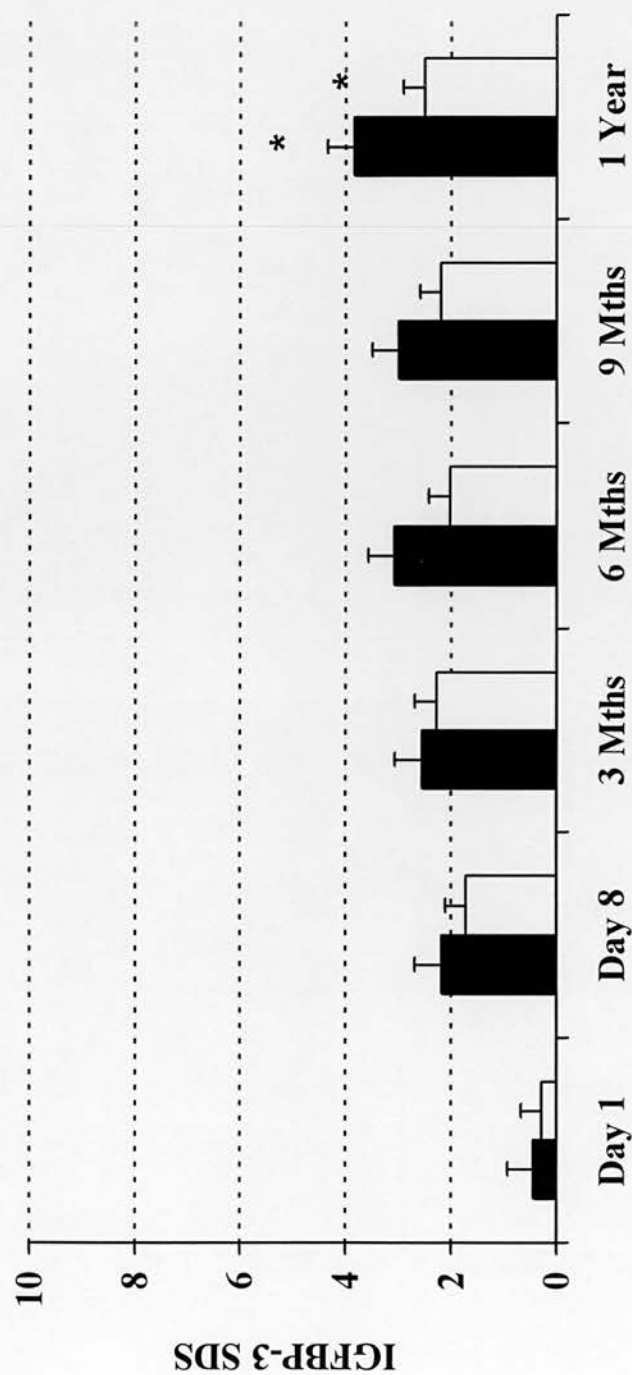


Figure 8.3 IGFBP-3 SDS before and during rhGH treatment in the CRI ■ and transplanted □ groups. IGFBP-3 SDS increased with rhGH treatment in both groups, * $p < 0.001$. Results are shown as the mean and SE.

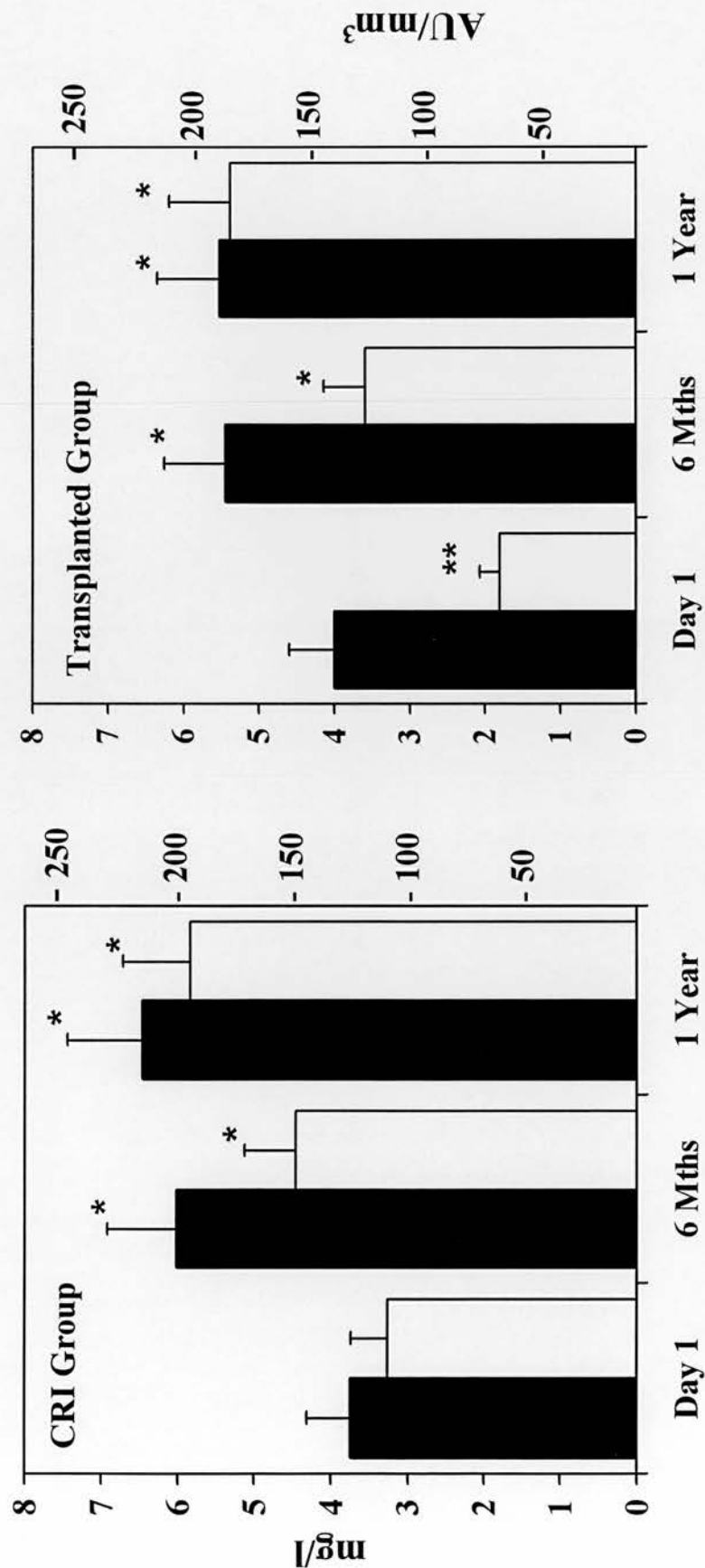


Figure 8.4 IGFBP-3 during one year of rhGH treatment in the CRI and transplanted groups. IGFBP-3 was measured by IRMA [■] and by Western ligand blot (WLB) [□]. IRMA measures total IGFBP-3; WLB measures intact IGFBP-3. Total IGFBP-3 by IRMA was similar in both groups on day 1, but intact IGFBP-3 was lower in the transplanted group compared with the CRI group, $p < 0.05$ vs CRI IGFBP-3 by both IRMA and WLB increased in both groups during rhGH treatment, $p < 0.001$ vs. Day 1

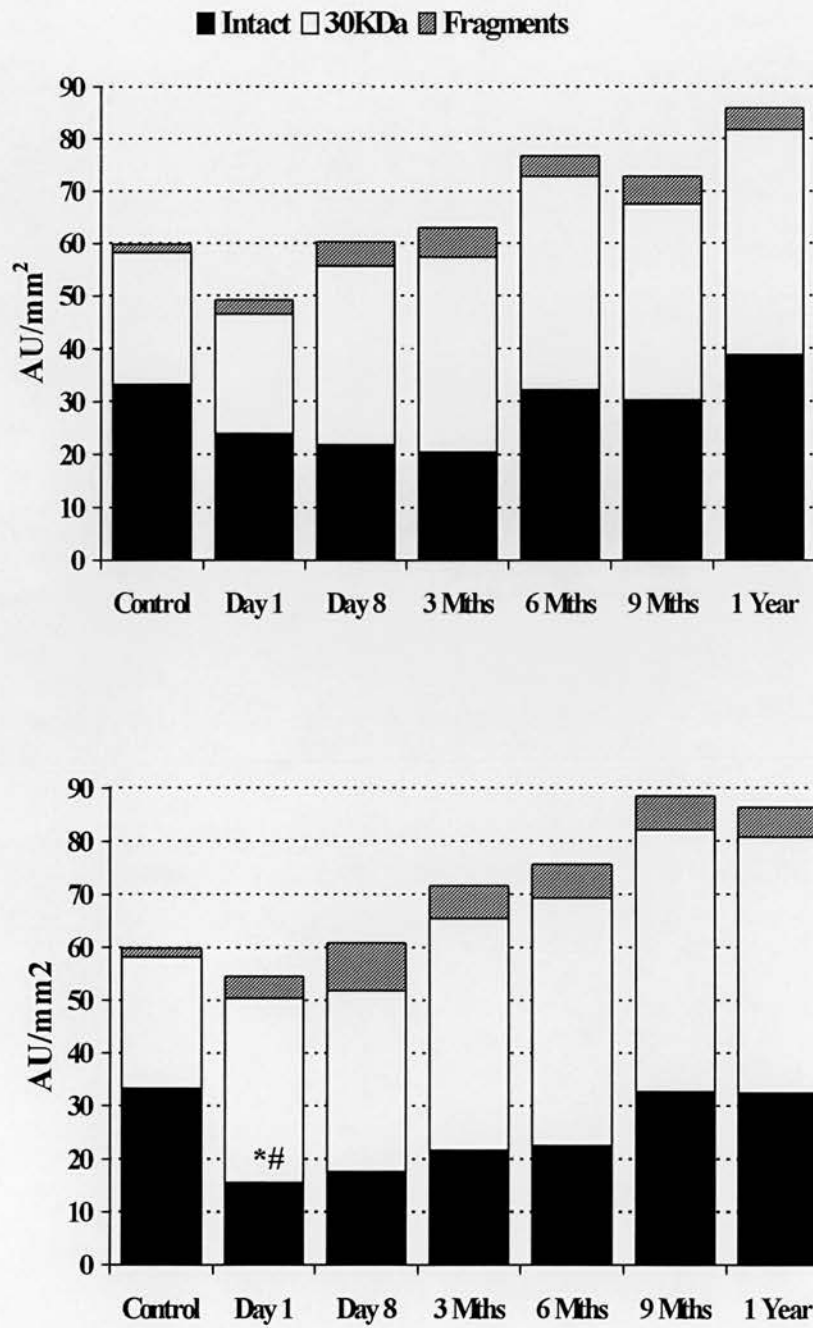


Figure 8.5 IGFBP-3 as measured by WIB. Total IGFBP-3 at each time point is composed of intact protein, a 30kDa fragment and smaller fragments. At the start of treatment the amount of intact IGFBP-3 was lower in the transplanted group compared with controls, * $p < 0.001$, and with the CRI group, # $p = 0.03$.

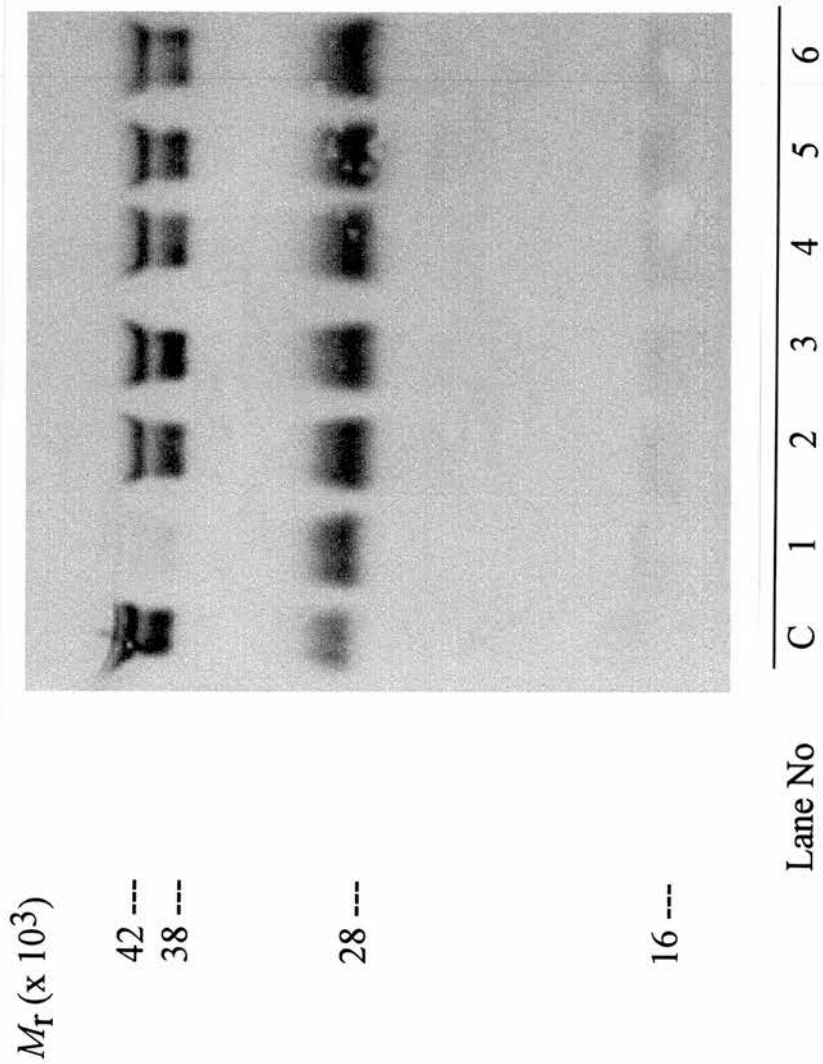


Figure 8.6 Representative western immunoblot from a transplanted patient. Lane C is pooled control sera. Lanes 1-6 show transplanted serum on day 1, day 8, and 3, 6, 9 and 12 months after starting rhGH. There was less intact IGFBP-3 (38 - 42 kDa) on day 1, and an increase in intact IGFBP-3 during rhGH treatment.

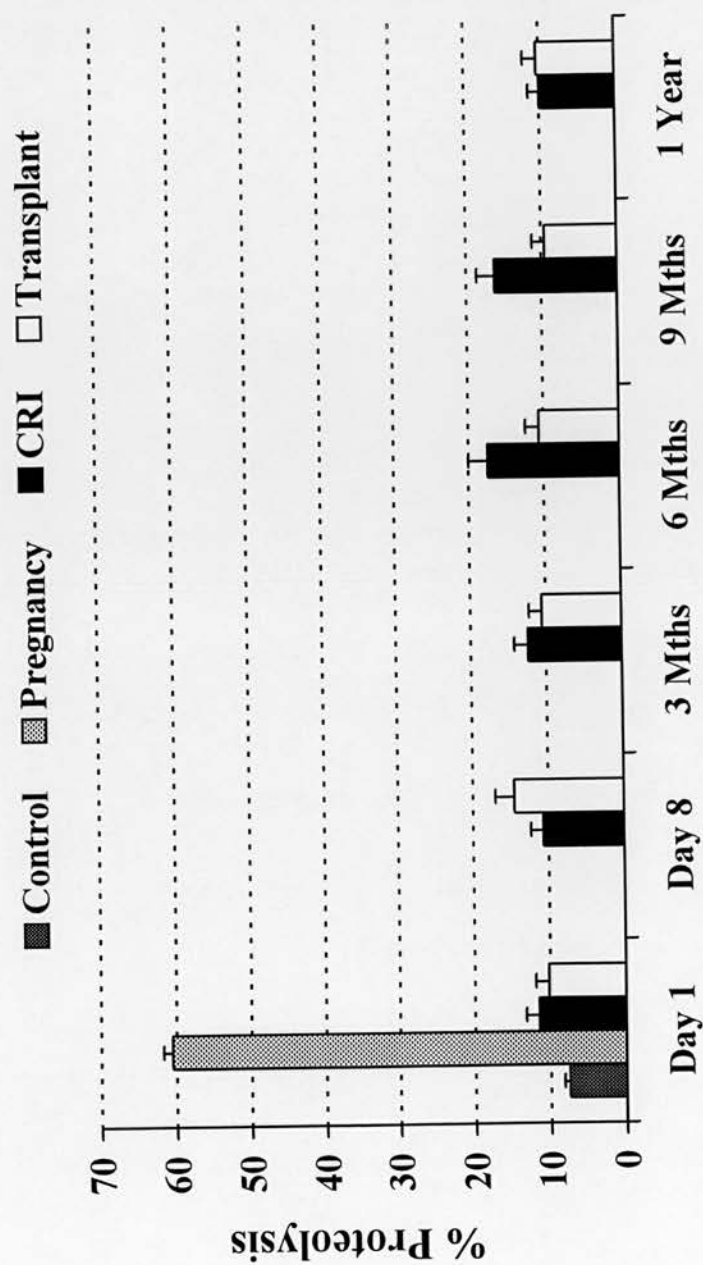


Figure 8.7 In vitro proteolysis in pooled control serum, term pregnancy serum, CRI and transplanted serum, before and during 1 year of rhGH therapy.

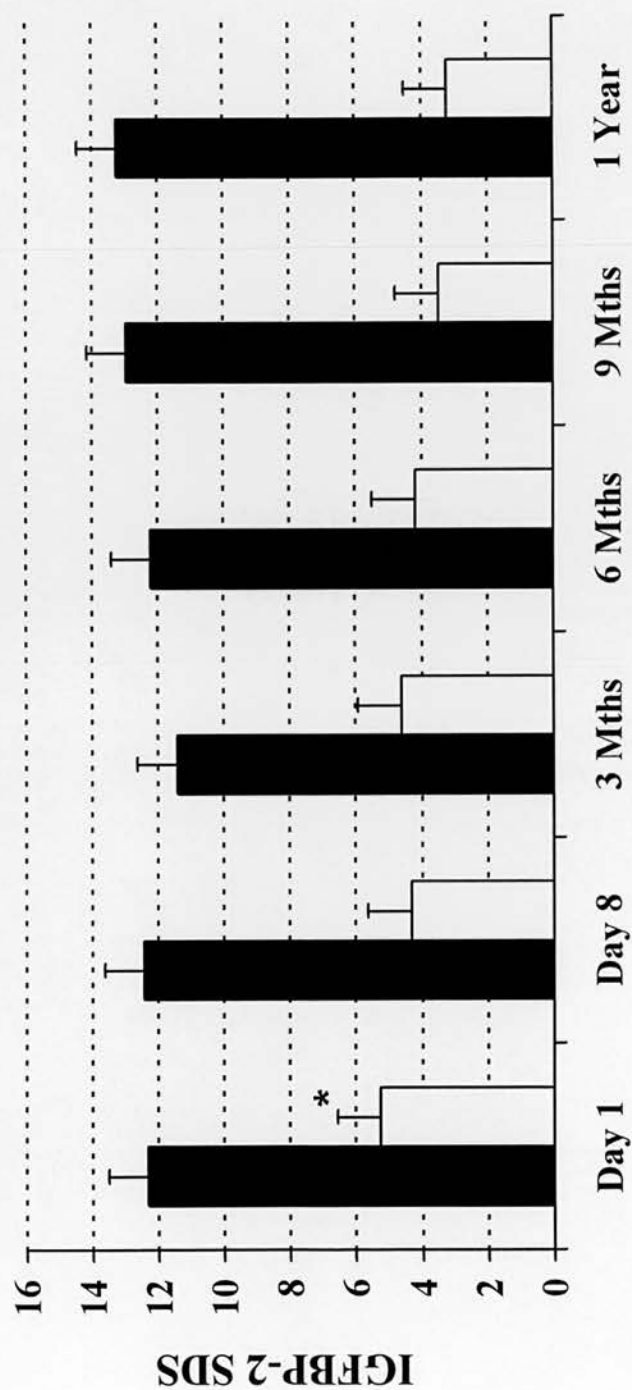


Figure 8.8 IGFBP-2 SDS before and during rhGH treatment in the CRI ■ and transplanted □ groups. IGFBP-2 was higher in the CRI vs. transplanted group on day 1, * $p = 0.006$. Results are shown as the mean and SE.

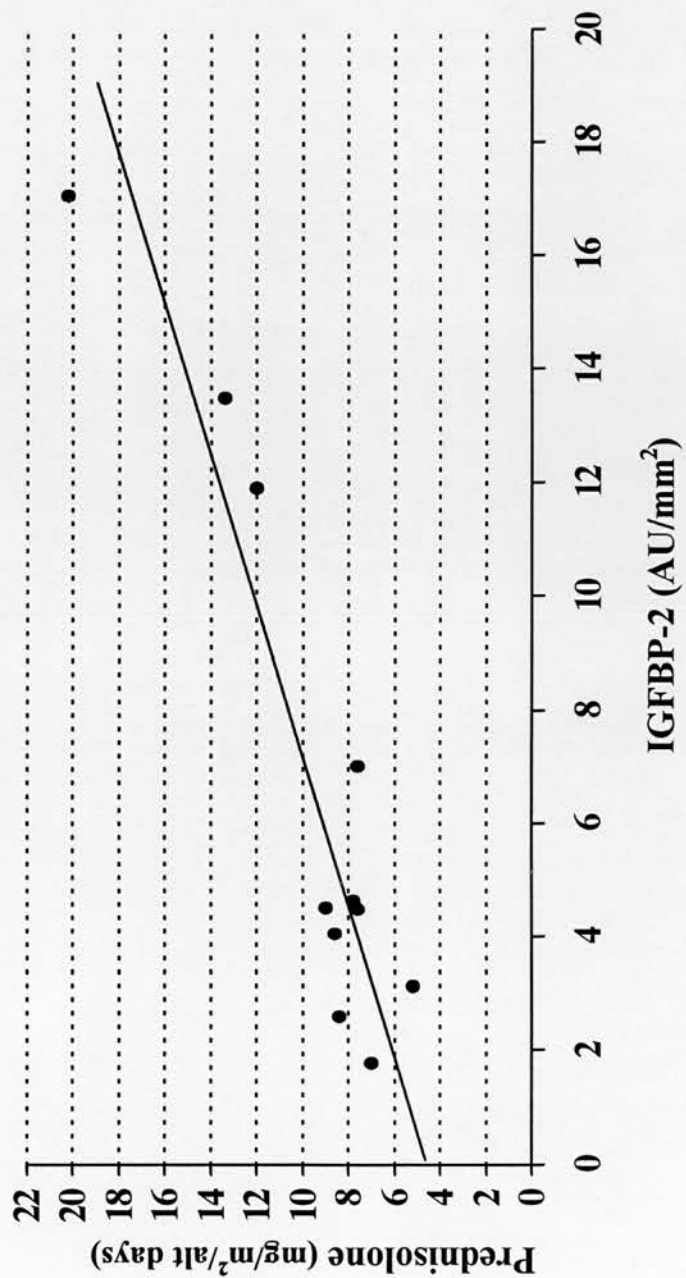


Figure 8.9 IGFBP-2, as measured by western ligand blot, plotted against prednisolone dose during rhGH treatment in the transplanted group, $r = 0.919$, $p < 0.0001$.

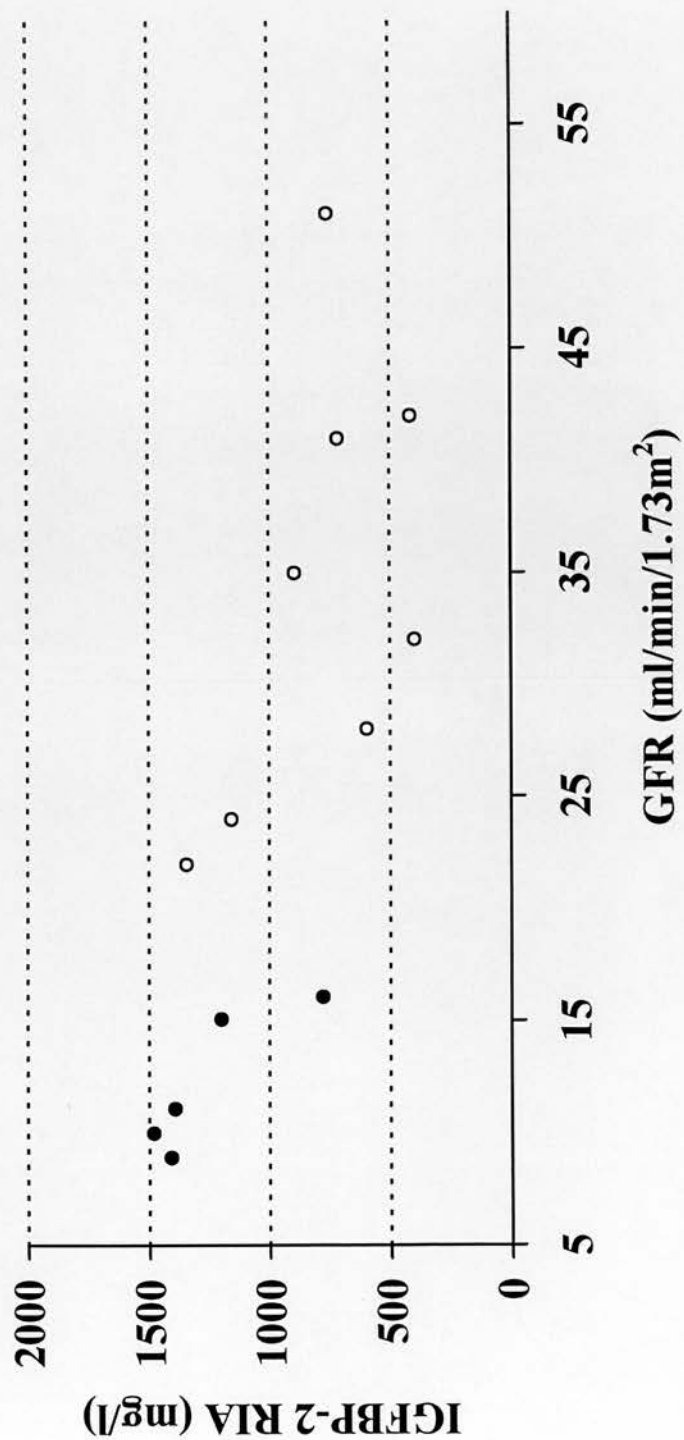


Figure 8.10 IGFBP-2 as measured by RIA plotted against GFR (ml.min/1.73m²) at baseline in the CRI ● ($r = -0.876$, $p = 0.05$) and transplanted ○ ($r = -0.624$, $p = 0.04$) groups.

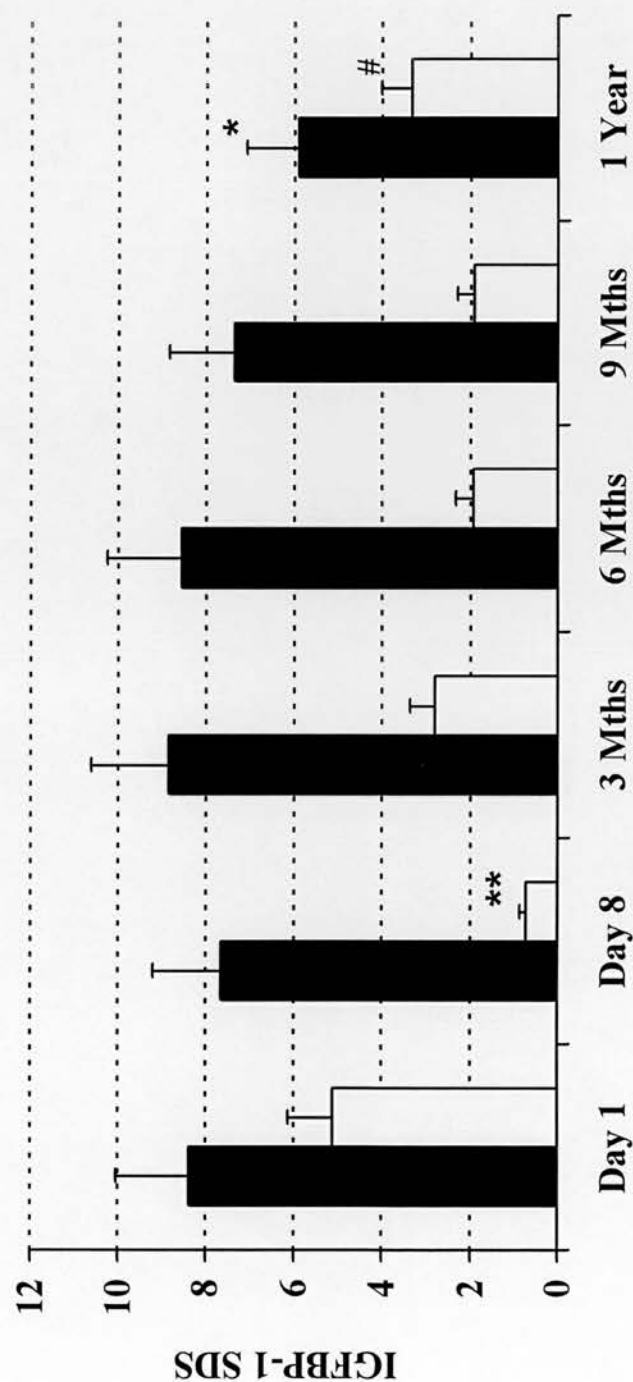


Figure 8.11 IGFBP-1 SDS before and during rhGH treatment in the CRI ■ and transplanted □ groups. In the CRI group, IGFBP-1 SDS decreased with rhGH treatment, * $p = 0.04$ ANOVA. There was a marked decrease in IGFBP-1 SDS after 1 week in the transplanted group, ** $p = 0.002$ ANOVA. IGFBP-1 SDS continued to fall during the year of treatment, # $p = 0.002$ ANOVA. Results are shown as the mean and SE.

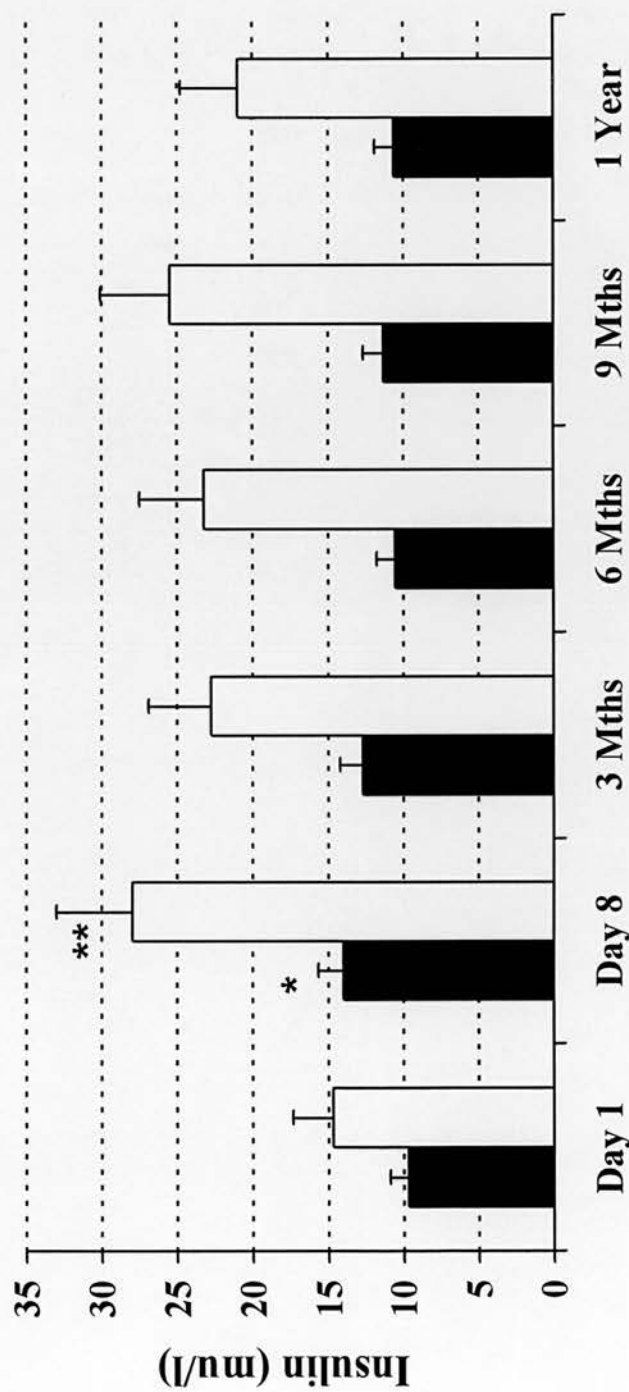


Figure 8.12 Insulin (mU/l) before and during rhGH treatment in the CRI ■ and transplanted □ groups. Insulin levels increased after 1 week in both the CRI group, * $p < 0.01$, and in the transplanted group, ** $p = 0.03$. Results are shown as the mean and SE.

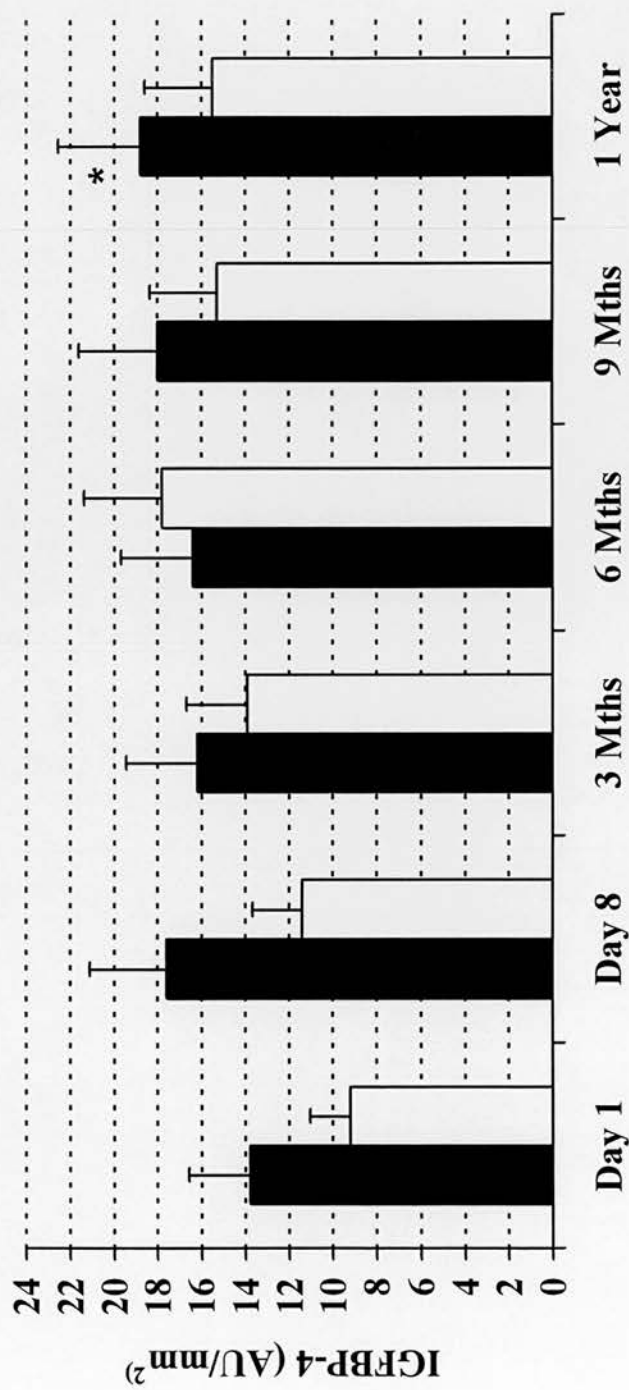


Figure 8.13 IGFBP-4 by western ligand blot before and during rhGH treatment in the CRI ■ and transplanted □ groups. IGFBP-4 increased with rhGH treatment in the CRI group, * $p = 0.06$. Results are shown as the mean and SE.

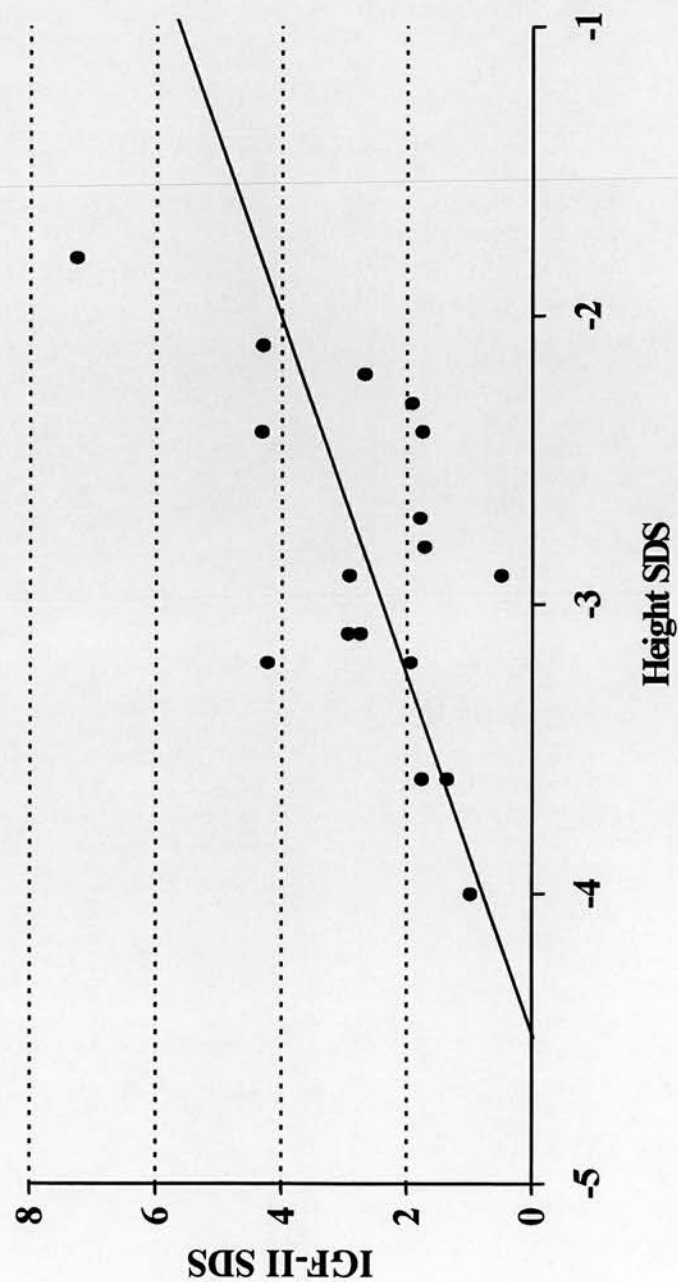


Figure 8.14 Height SDS at the start of treatment plotted against IGF-II SDS on day 1 in the CRI group, $r = 0.593$, $p = 0.01$.

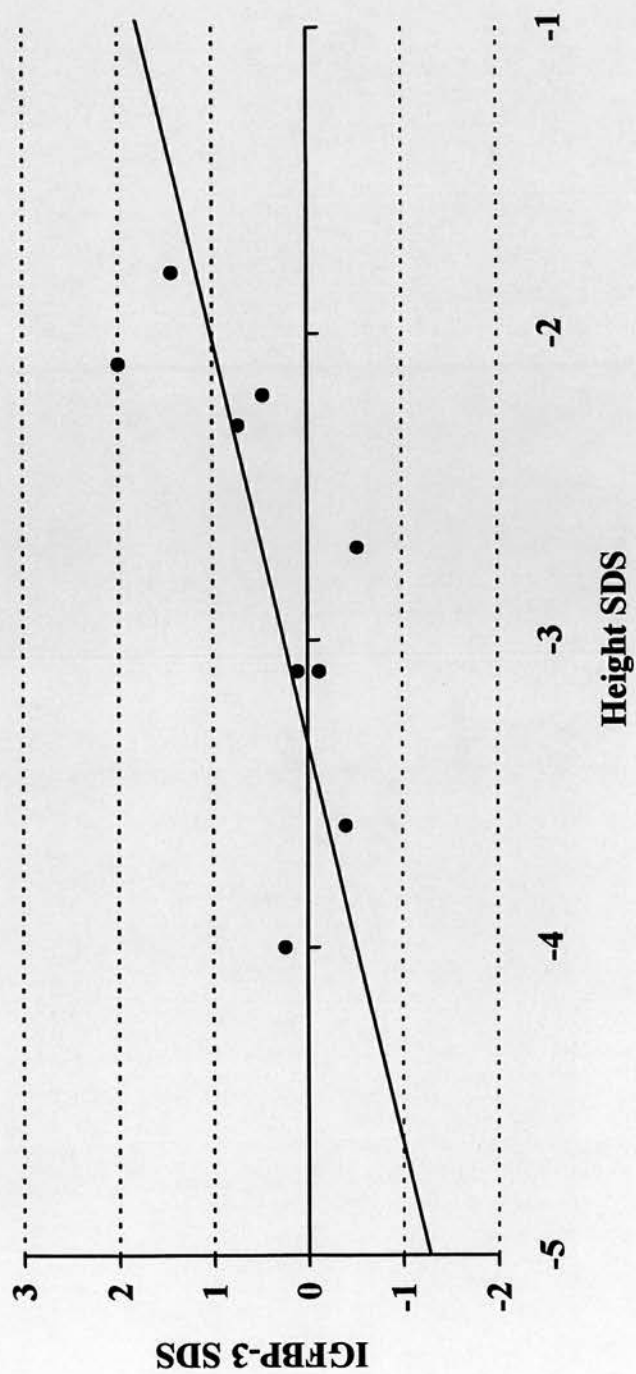


Figure 8.15 Height SDS at the start of treatment plotted against IGF-II SDS on day 1 in the CRI group, $r = 0.683$, $p = 0.04$.

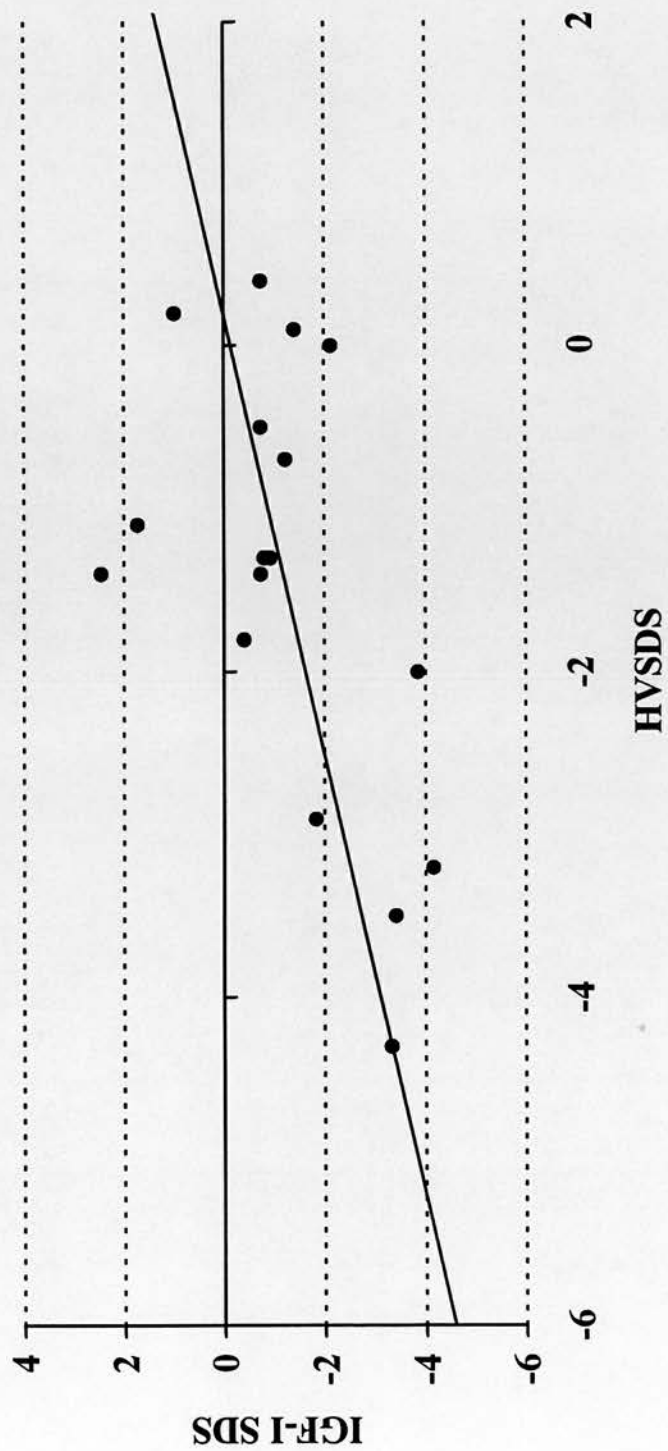


Figure 8.16 HVSDS in the year before treatment plotted against IGF-I SDS on day 1 in the CR1 group, $r = 0.551$, $p = 0.02$.

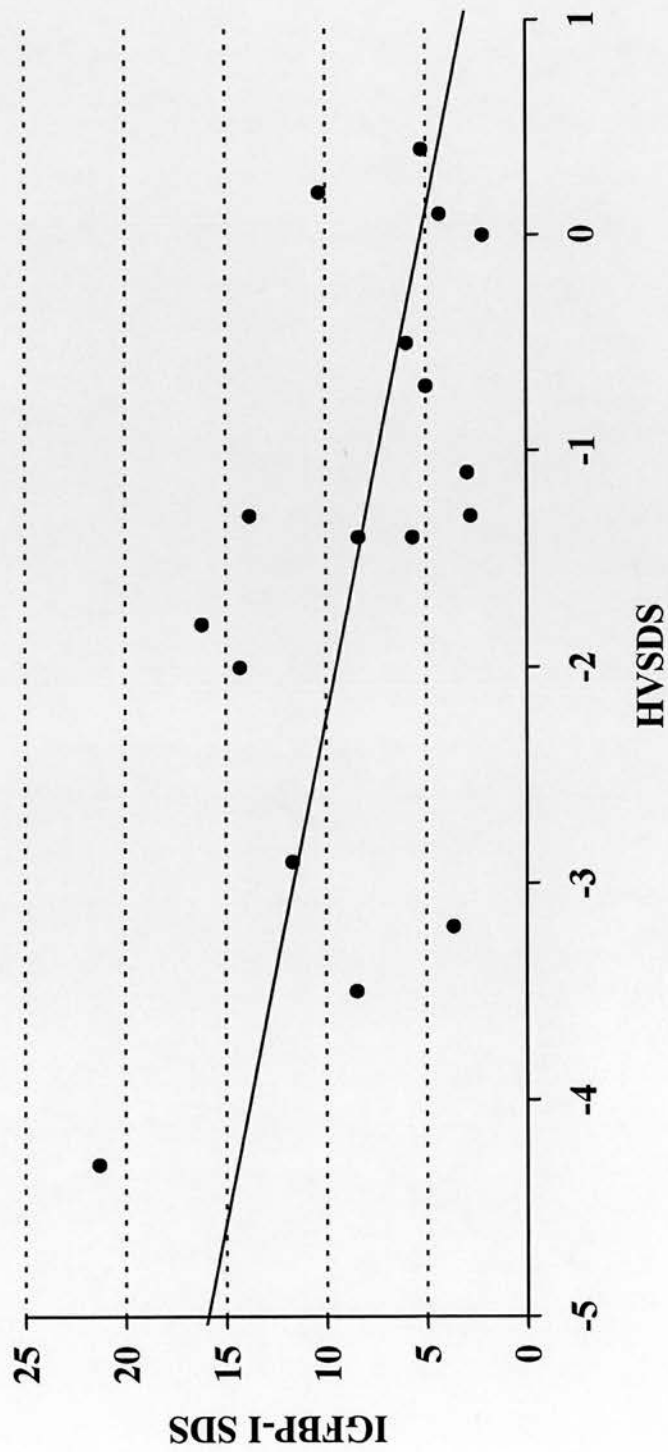


Figure 8.17 HVSDS in the year before treatment plotted against IGFBP-I SDS on day 1 in the CRI group,
 $r = -0.535, p = 0.03$

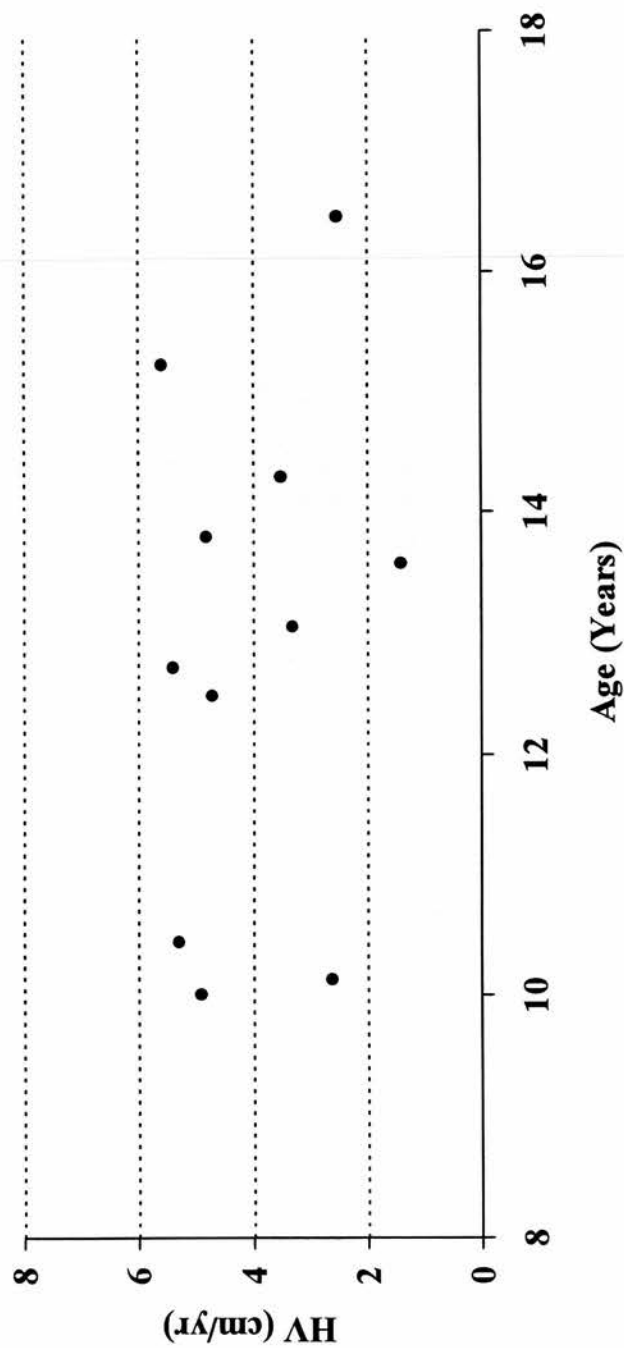


Figure 8.18 Height velocity (HV) in the transplanted group in the year before rhGH treatment was unrelated to age, $r = -0.202$, $p = 0.55$.

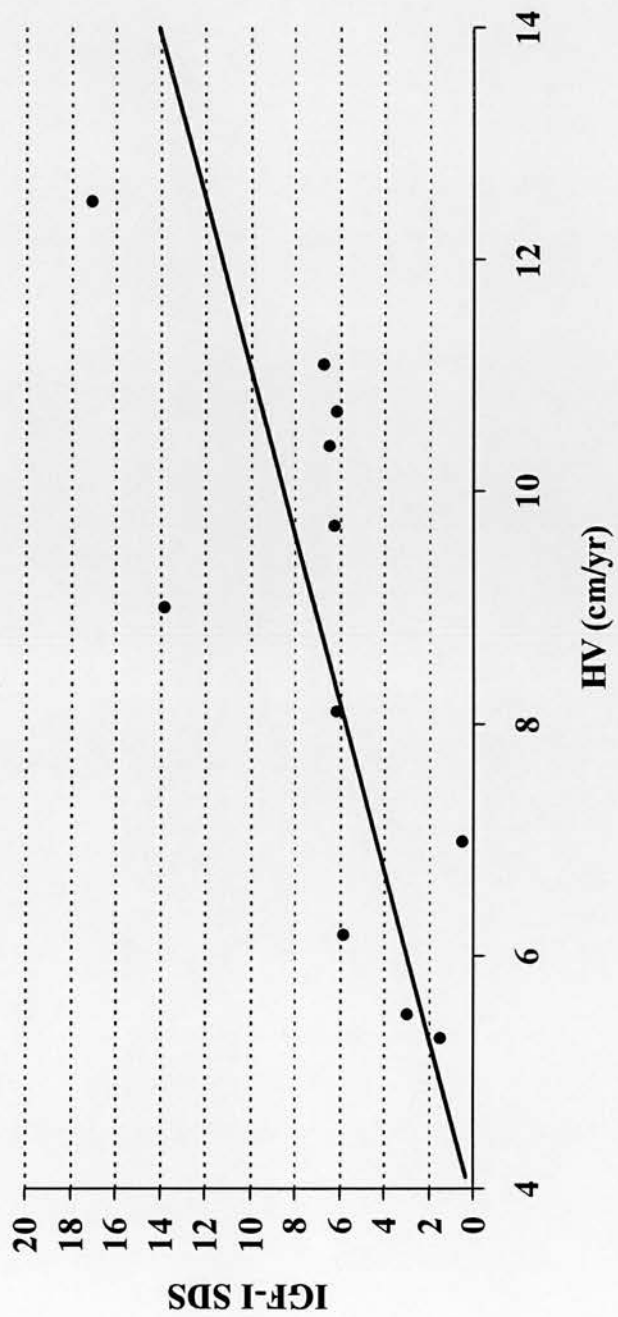


Figure 8.19 Height velocity (HV) in the transplanted group during the year of rhGH treatment plotted against IGF-I SDS, $r = 0.692$. $p = 0.02$.

Chapter 9: The Effects Of Recombinant Human Growth Hormone On The Immune System In Children With Chronic Renal Insufficiency and Renal Transplants

Introduction

It has long been recognised that there is a link between pituitary hormones and the immune system. As early as 1930 Smith demonstrated that ablation of the rat anterior pituitary gland resulted in regression of the thymus gland (Smith 1930), and nearly forty years later, Pierpaoli showed that the hormone responsible was GH; antiserum to GH injected into mice with intact pituitary glands also caused thymic atrophy (Pierpaoli *et al.* 1968). It is also well recognised that GH is necessary for certain lymphocyte functions *in vitro* (Snow *et al.* 1981), and in animals, experimentally induced hypopituitarism results in impaired immune function, which can then be restored with GH replacement (Baroni *et al.* 1969; Dusquessnoy *et al.* 1972).

In contrast, with rare exceptions only (Monafo *et al.* 1991, Fleischer *et al.* 1980, Tang *et al.* 1993), children with GHD are not immunocompromised and have normal indices of immune function (Abassi and Bellanti 1985; Rapaport *et al.* 1986; Etzioni *et al.* 1988; Bozzola *et al.* 1989; Petersen *et al.* 1990; Rapaport *et al.* 1991; Spadoni *et al.* 1991). Growth hormone replacement has been reported to decrease transiently the % B cells and CD4⁺ cells, although not to subnormal levels (Rapaport *et al.* 1986; Petersen *et al.* 1990; Rapaport *et al.* 1991; Spadoni *et al.* 1991). On the other hand, some authors report no change in lymphocyte subsets or immune function (Abassi and Bellanti 1985; Etzioni *et al.* 1988; Bozzola *et al.* 1989).

Immune function is altered in CRF, although the majority of children are not clinically immunocompromised (Drachman *et al.* 1989). Potentially this could be aggravated by rhGH treatment. Children with renal transplants receive up to three immunosuppressant medications, and clearly are immunocompromised, particularly in the early post transplant period. Any alteration in immune function could potentially

increase the risk of rejection of the graft (Tyden *et al.* 1990; Schwartz and Warady 1992), or result in an increased incidence of infection.

We performed immunological studies in two subgroups of children in the BAPN multi-centre trial of rhGH in CRF (Maxwell *et al.* 2000). Lymphocyte subsets and markers of T cell activation were measured before and during rhGH treatment. Haematological indices were also measured to determine if rhGH treatment affected red cell, platelet or granulocyte numbers.

Twenty-one children in the BAPN trial of rhGH in CRF were studied. These patients were being treated in the Paediatric Nephrology departments at the Royal Free and Guy's Hospitals, London. Eleven children (2 girls) had chronic renal insufficiency and were being managed conservatively (CRI); 10 (3 girls) children had renal transplants.

Patients

Patient details are given in Table 9.1. GFR was less than 50 ml/min/1.73m² in the CRI group, and had been so for at least one year. The transplanted patients had stable graft function, and had received their grafts more than one year previously; the mean time from transplantation being 5.2 (1.9 - 10.2) years. Immunosuppression consisted of prednisolone at a mean (range) dose of 10.2 (7.6 - 20.2) mg/m²/alternate day, azathioprine (60 mg/m²/day) and in 7 children, cyclosporin A at a dose sufficient to maintain plasma 12 hour trough levels between 50 - 150 ng/ml. No child in either group received recombinant erythropoietin.

Methods

Lymphocyte subsets, T cell activation markers and full blood counts were measured. The transplanted children were part of a controlled trial, and therefore had up to 1 year of control data before starting rhGH treatment. Less pre-treatment data were

available for the CRI group, so the data for the two groups were analysed in a different fashion.

The children were studied on between 1 and 4 occasions in the year before starting rhGH (control phase). After commencing rhGH they were seen after 1 week, then at 3 monthly intervals for 1 year (rhGH phase). At each visit the number of episodes of illness, courses of antibiotics, and rejection episodes were recorded. For the purposes of the study, a rejection episode was diagnosed by the managing clinician and was defined as the need for treatment with high dose oral prednisolone for 3 days.

Height was measured using a Harpenden stadiometer. Glomerular filtration rate was measured by clearance of inulin on day 1 and after 1 year (Maxwell *et al.* 1995).

At each visit haemoglobin, platelets, total and differential white counts were measured. Five ml of blood was collected in lithium heparin tubes for estimation of lymphocyte subsets and markers of T cell activation. The % and absolute number of lymphocytes, T (TCR $\alpha\beta$) cells, CD4⁺, and CD8⁺ cells, B cells and natural killer (NK) cells were calculated. The number of and % of T cells expressing the Il-2 receptor (CD25), major histocompatibility complex class II antigens (HLA-DR) and another marker of T cell activation, CD26 (dipeptidylpeptidase), were recorded. These 3 antigens increase during T cell activation at early, intermediate and late times respectively. Episodes of illness, graft rejection (as diagnosed by the clinician in charge) were recorded.

Lymphocytes were separated on Lymphoprep (Nygaard, Denmark) and incubated with T10B9 (TCR $\alpha\beta$ receptor) together with a second antibody (listed below). Staining was performed by double fluorescence flow cytometry (Facsan flowcytometer, Becton Dickinson) using anti-mouse IgM^{PE} and anti-mouse IgG^{FITC} as second layers. Using the lymphocyte gate determined by forward angle and side scatter, the % total T cells (TCR $\alpha\beta$), T_H (CD4⁺), T_c (CD8⁺), B (CD19⁺) and NK (CD16⁺CD7⁺) cells were determined. The absolute number of each lymphocyte subset

was then calculated from the paired total lymphocyte count. The % T cells expressing CD25, CD26 and HLA-DR antigens were determined.

Antibodies

Monoclonal antibodies against CD2 (RFT11), CD4 (RFT4), CD7 (RFT2), CD8 (RFT8), CD25 (RFT5), HLA-DR (RFTDR2) were raised in the Department of Immunology, Royal Free and University College Medical School. The following antibodies were also used: T10B9 (TCR $\alpha\beta$) a kind gift from Professor Thompson, Kentucky, USA); CD16 (Leu11b, Becton Dickinson); CD26 (Ta-1, Coulter).

Statistical Analysis

As lymphocyte counts vary with age, and because it was not possible to find a control group exactly matched for age, sex, underlying disease, time from transplantation, donor source, renal function and medication, the patients acted as their own controls.

In the CRI group, change in the parameters studied was assessed by ANOVA. Statistical significance was further tested by calculating the confidence interval of the difference of the mean value at each time point and that on day 1. If the confidence interval of the difference in means excluded zero, then statistical significance was confirmed.

Data during rhGH treatment in the transplanted group were analysed in a similar fashion, but in this group data were also available from the year before rhGH treatment (control phase). To compensate for day to day variation, the average value of each subset or activation marker was calculated during both the control and rhGH phases, and these 2 sets of results were compared using a paired Student's *t*-test. To exclude a difference due to the effects of time alone, the change in lymphocyte numbers and activation markers during each phase was also calculated and compared by a paired Student's *t*-test. The summary data are expressed as the mean \pm SE. Again

statistical significance was further tested by calculating the confidence interval of the difference of the mean value at each time point and that on day 1.

A p value of < 0.05 was taken to represent statistical significance. The number of rejection episodes, dose of prednisolone and time since transplantation were correlated both with the number of cells of each lymphocyte subset, and the % of T cells expressing activation markers, using multiple regression analysis.

Results

In the CRI group, 10 of the 11 children completed the study; 1 child reached end stage renal failure and required dialysis 9 months into the study. Only data prior to starting dialysis have been used for analysis in this child. Mean HV improved during the year of rhGH treatment, from 4.1 (1.7 - 6.3) to 9.3 (5.9 - 12.5) cm/yr, $p < 0.001$. There was no change in GFR during the study: 18 (9 - 59) on day 1 and 19 (5 - 59) ml/min/1.73m² at 1 year.

In the transplanted group, 9 of the 10 children completed one year of treatment; one patient returned to dialysis 11 months after entering the study. Only data relating to the first 9 months of rhGH treatment in this patient have been included. Height velocity increased from 4.2 (2.6 - 5.6) cm/yr in the year before rhGH to 8.5 (5.3 - 11.7) cm/yr during treatment, $p < 0.001$. Glomerular filtration rate remained unchanged; 61 (26 - 93) on day 1 and 61 (24 - 109) ml/min/1.73m² at 1 year. There were no serious infective episodes during treatment, and no increased incidence of infections compared with the year before the trial.

During the year of rhGH treatment, 5 children had 9 episodes of presumed rejection. None of these episodes coincided with study visits. No anti-lymphocyte preparations were given. These same 5 children had 12 such episodes in the year preceding rhGH, giving rejection rates for all 10 children of 1 per 10 patient months in the year before rhGH and 1 per 13 patient months during rhGH. These rates were not statistically

different. The other 5 children had no episodes of graft dysfunction before or during treatment.

I. Haematological Indices

Haemoglobin, total and differential white cell counts and platelet numbers were measured in each group. Comparison of the baseline values in each group is shown in Figure 9.1. The only difference between the groups was in the lymphocyte count, which was significantly lower in the transplanted group, $p = 0.05$.

During rhGH treatment there was a decrease in haemoglobin in the CRI group, but there were no other significant changes, Figure 9.2. The data are also shown in Table 9.2a for the CRI group and 9.2b for the transplanted group. In addition for the transplanted group, the averaged values during the control and treatment phases are shown in Figure 9.3, and Table 9.3. The mean (SE) changes during both the control and treatment phases are shown in Table 9.4. There was no difference between the changes seen before and those seen during rhGH treatment.

II. Lymphocyte Subsets

The total lymphocyte count, T cells, $CD4^+$, $CD8^+$, B cells and NK cells in each group are shown in Tables 9.3 - 9.5, and Figures 9.2 - 9.8. Values at baseline in each group are shown in Figure 9.3.

CRI Group

During treatment there was no change in the total number of lymphocytes, but there was a significant decrease in the number of T cells (ANOVA, $p=0.03$). This was due to a decrease in the number of $CD4^+$ T cells ($p=0.04$), with no significant change in $CD8^+$ cells ($p=0.57$). There was no significant change in the number of B cells

($p=0.13$), or in the number of NK cells ($p=0.14$). Summary data are shown in Table 9.5a, and individual patient data are shown in Figures 9.4 and 9.5.

Transplanted group

Summary data for the children in the transplanted group during rhGH treatment are shown in Table 9.5b. Individual values are shown in Figures 9.6 and 9.7.

Comparison of the control and treatment phases is shown in Figure 9.8 and the changes during the control and treatment phases are given in Table 9.4. There was no significant difference in the absolute number of total lymphocytes, T cells, CD4⁺ or CD8⁺ cells, between the control and rhGH phases. The B cell count was lower during rhGH treatment than during the control phase (control: 0.11 (0.07); rhGH: 0.07 (0.06), $p = 0.015$) (Figure 9.8), but there was no significant difference between the change in B cell number during each phase ($p = 0.72$), suggesting an effect due to time rather than rhGH (Table 9.3). The average T_H / T_C ratio in the control phase did not differ from that in the rhGH phase: 2.32 (0.32) vs. 2.41 (0.53), $p = 0.91$.

There was no relationship between lymphocyte numbers and rejection episodes, GFR, or prednisolone dose. There was a trend for a negative correlation between the total number of lymphocytes and the length of time since transplantation ($r = -0.569$, $p = 0.08$).

The 5 children who had rejection episodes before and during rhGH treatment, were compared with the 5 who had no rejection episodes during the 2 years of study. There was no significant difference between these 2 groups either before or during rhGH treatment in terms of the number of each type of lymphocyte subset. There was a trend for higher CD4 (T_H) counts during rhGH treatment in the children with no rejection episodes ($p = 0.06$).

III. T Cell Activation Markers

Surface expression of CD25, CD26 and of HLA-DR antigens showed wide variation at the start of treatment in both groups. Summary data are given in Tables 9.3, 9.4 and 9.6, and Figures 9.3 and 9.8. Individual values are shown in Figures 9.9 and 9.10. Mean and median values for CD25 and HLA-DR were similar in both groups. There was increased expression of CD26 in the transplanted group as compared to the CRI group (Figure 9.3).

CRI Group

Summary data during rhGH treatment are shown in Table 9.6a, and individual patient values are depicted in Figure 9.9. There was no significant change in T cell expression of CD25 or HLA-DR antigens during rhGH treatment. There was increased expression of CD26 at 3 and 6 months, but not at 1 year. Over the year of treatment, the change was not significant ($p = 0.11$). As the number of T cells was reduced during treatment, there was no change in the absolute number of T cells expressing CD26 ($p = 0.53$).

Transplanted Group

Expression of activation markers during rhGH treatment in the 10 transplanted children is shown in Table 9.6b and Figure 9.10. The averaged values of the number of T cells expressing activation markers during the control and treatment phases are given in Figure 9.8.

In the transplanted group surface expression of CD25 was varied, such that at times during the control phase, between 1 and 42 % of T cells expressed CD25. The average expression of CD25 was lower during rhGH treatment than during the control phase. There was however no significant difference between the change in % T cells expressing CD25 in the control phase compared with the rhGH phase ($p = 0.31$), suggesting an effect of time alone. There was no significant change in

expression of CD26 antigen nor of HLA-DR antigens on T cells during either the control or treatment phases.

Expression of activation markers was unrelated to the number of rejection episodes, either before or during rhGH treatment. There was no difference between the expression of CD25, CD26 and HLA-DR antigens between the groups with or without rejection episodes, either before or during rhGH treatment. During the control phase, there was no relationship between expression of CD25, CD26 or HLA-DR antigens and the time from transplantation nor the dose of prednisolone.

Discussion

Concern surrounding the use of rhGH in CRF has arisen from the recognition that there are links between GH and the immune system, both during development and in post natal life. The multitude of experimental evidence which has accumulated since Smith's original work, where he demonstrated involution of the thymus in the rat following ablation of the anterior hypophysis (Smith 1930), attests to the existence of bi-directional communication between GH and the immune system.

It is well known that GH stimulates immune function *in vitro* (Snow *et al.* 1981), and the link between GH and the immune system is strong in small mammals (Fabris *et al.* 1971). The dwarf Snell mouse, which has congenital deficiency of the pituitary due to a mutation in the Pit-1 gene, has arrested development of the thymus and marked T cell immunodeficiency (Baroni *et al.* 1969; Duquessnoy *et al.* 1972). Growth hormone replacement not only improves growth but also restores immune competence, and prevents involution of the thymus and depletion of peripheral lymphoid tissues. Lymphocyte, erythrocyte, neutrophil and platelet counts are increased with GH replacement (Murphy *et al.* 1992). Mice which are transgenic for GH have enlarged lymphoid organs (Clark *et al.* 1997).

In man the link is less strong but peripheral lymphocytes express GH (Kiess *et al.* 1985) and IGF-I (Eshet *et al.* 1975) receptors, and *in vitro* studies indicate a role for GH in lymphopoiesis (Snow *et al.* 1981), granulopoiesis (Merchaw *et al.* 1988) and erythropoiesis (Golde *et al.* 1977), and in granulocyte (Weidemann *et al.* 1991) and macrophage (Edwards *et al.* 1988) function. Growth hormone has been shown to stimulate DNA synthesis in T lymphocytes (Gelato *et al.* 1993), and to stimulate proliferative and cytotoxic responses in mixed lymphocyte culture (Snow *et al.* 1981). Natural killer cell function is increased by GH (Crist *et al.* 1987, 1990). Growth hormone enhances the production of Il-2 and Il-6 by lymphocytes (Berczi *et al.* 1997). While most reports suggest a stimulatory role for GH and the immune system, others indicate an inhibitory effect (Kiess *et al.* 1983). Some of the effects of GH are mediated by local production of IGF-I (Geffner *et al.* 1990).

Despite the evidence linking GH and the immune system, GHD in man is not associated with reduced immunity, with the rare exception of case reports of families with GHD and agammaglobulinaemia (Monafo *et al.* 1991), hypogammaglobulinaemia (Fleisher *et al.* 1980), and also with combined immunodeficiency (Tang *et al.* 1993). Most children with GHD have normal indices of immune function (Abassi and Bellanti 1985; Rapaport *et al.* 1986; Etzioni *et al.* 1988; Bozzola *et al.* 1989; Petersen *et al.* 1990; Rapaport *et al.* 1991; Spadoni *et al.* 1991), although there are occasional reports of a decrease in Il-2 production (Casanova *et al.* 1990), a decrease in NK activity (Crist *et al.* 1987; Kiess *et al.* 1988; Bozzola *et al.* 1990) and decreased B cells in GHD (Gupta *et al.* 1983).

Most patients with GHD do not have complete absence of GH. However even patients with the Pit-1 gene mutation, who do have complete GHD, have no evidence of immunosuppression (Wit *et al.* 1989). Furthermore, GH and IGF-I (and prolactin) are also secreted by cells of the immune system in an autocrine fashion (Weigent *et al.* 1991a; Baxter JB *et al.* 1991). So complete absence of GH is rare. There is also overlap or redundancy between the actions of different hormones on the immune system such that absence of one may not be clinically evident. Therefore in children

with GHD, there would appear to be subtle and variable changes in laboratory parameters of immune function, but no clear evidence of clinical immunodeficiency.

There is also no clinical evidence in man that administration of exogenous GH affects the immune system *in vivo*. The changes in lymphocyte subsets that are seen with GH therapy in GHD appear to be minor. Some groups report a transient reduction in % B cells and % T helper cells (Rapaport *et al.* 1986; Petersen *et al.* 1990; Rapaport *et al.* 1991; Spadoni *et al.* 1991), although not to subnormal levels. Other groups report no change in lymphocyte subsets during GH treatment (Abassi and Bellanti 1985; Etzioni *et al.* 1988; Bozzola *et al.* 1989), but an increase in mitogen stimulated lymphocyte proliferation has been reported (Abassi and Bellanti 1985).

In CRF the immune system has been variously reported to be normal or depressed. One study in children with CRF found lymphocyte subsets and mitogen-stimulated proliferative responses to be normal, although % B cells was lower than controls (Drachman *et al.* 1989). This is in contrast to our findings; the number and % of B cells were well within the normal range for our laboratory. Mean GFR in this study and that of Drachman, was similar. The same authors report a normal % B cells in children on dialysis. Whilst this might suggest a dialysable inhibitor of B cells in CRF, one would then expect a relationship between B cells and GFR. This did not exist in their study nor was it present in our study.

Other workers describe a state of chronic activation of the immune system in uraemia, so-called preactivation, with increased expression of IL-2 receptors on T cells and an increase in soluble IL-2 receptors (shed from the cell surface after binding of IL-2 to its receptor on activated T cells) (Beaurain *et al.* 1989). This has not been confirmed by all groups (Caruana *et al.* 1992). Indeed in our patients IL-2 receptor expression was not increased. There was no change with rhGH treatment.

There are few published data looking at the effect of rhGH on lymphocyte subsets or activation markers in CRI and renal transplantation. Our finding of a reduction in the

number of CD4⁺ cells in the CRI group is interesting as the same effect has been reported with rhGH in GHD (Rapaport *et al.* 1986; Petersen *et al.* 1990; Rapaport *et al.* 1991; Spadoni *et al.* 1991). In over 90% of the CRI children however the CD4 count remained within the normal range. The B cell count was not affected. There was no increased incidence of infections, so the clinical significance for the CRI group seems slight. In contrast to the other reported studies, we expressed our results as lymphocyte numbers rather than % subsets, although both variables were calculated. The total number of lymphocytes was unchanged, therefore the % of T cells and CD4⁺ cells also decreased. The lack of a control group for this group of patients means that it is impossible to exclude that the reduction is due to an effect of time alone. There was no significant change in the number of lymphocyte subsets in the transplanted children, however the number of CD4⁺ cells at the start of treatment was significantly lower in the transplanted group compared to CRI group, and may explain the difference.

The findings of three recent publications taken together suggest a potential mechanism for the rhGH induced changes in lymphocyte subpopulations that have been reported in GHD. It has been known for some time that lymphocytes have receptors for GH (Eshet *et al.* 1975) and are also able to secrete GH (Weigent *et al.* 1991b). More recently flowcytometry has demonstrated that B cells have a higher expression of GH receptors than T or NK cells (Badolato *et al.* 1994). Secondly in cultures of rat spleen cells, secretion of GH is seen primarily by B cells, T helper cells and macrophages, while T suppressor cells and NK cells produced only small amounts of GH (Weigent *et al.* 1991a). Thirdly addition of rhGH down-regulates the number of receptors on cultured lymphocytes in a dose-dependent fashion (Smal *et al.* 1985). Taking these observations together, GH treatment may reduce the number of GH receptors on lymphocytes and therefore decrease GH induced proliferation, be that exogenous or endogenous GH. This sequence of events would predominantly affect B cells and T helper cells. The lack of effect on B cells in our patients might reflect a pre-existing effect of the underlying condition or previous treatment on B cell numbers or function.

The possible consequence of rhGH treatment in transplanted children are of an increase in the incidence of infection or rejection. To date there has been no reported increase in infections with rhGH, but the risk of rejection remains unclear. There are a number of published studies of rhGH post renal transplantation. There has been no reported increase in serious infections, but there have been occasional reports of actual or presumed rejection episodes after starting rhGH treatment (Tyden *et al.* 1990; Schwartz and Warady 1992). Other studies show no effect on the incidence of rejection (Bartosh *et al.* 1992; Fine *et al.* 1992; Tonshoff *et al.* 1993; Ingulli *et al.* 1993; Jabs *et al.* 1993; Benfield *et al.* 1993; Janssen *et al.* 1993; Hokken-Koelega *et al.* 1994d).

The overall finding in this, the BAPN trial, was that there was no increase in the incidence of rejection during rhGH treatment (Maxwell *et al.* 1998b, Chapter 6). The Dutch multi-centre study also showed no overall increase in the incidence of rejection, but suggested that those patients on alternate day steroid were more at risk of rejection episodes than those receiving daily steroids (Hokken-Koelega *et al.* 1994d). All of our patients received alternate day steroids. The most recent and largest multi-centre study to be published is that on behalf of the French Society of Pediatric Nephrology. Whilst overall there was no increased incidence of rejection in those treated with rhGH compared to controls, there was an increased risk of rejection in those with a history of 2 or more rejection episodes in the year before treatment. Six of 17 children with a history of a previous rejection episode had a further episode during rhGH treatment compared with only 1 of 21 such patients in the control group, $p=0.01$ (Guest *et al.* 1998). In our study, only those children with clinically diagnosed rejection episodes in the year before rhGH treatment had further rejection episodes during treatment.

Many of those patients who had rejection episodes would have chronic rejection or chronic allograft nephropathy. Consequently they will have a lower GFR and less renal reserve, and are therefore more likely to have fluctuations in creatinine levels. It is impossible to know if rhGH is causing 'rejection', or if increased body muscle mass

is responsible for the elevation in creatinine. Some children undoubtedly had acute rejection diagnosed by biopsy, but in others the exact mechanism of graft dysfunction was unknown.

Of interest however is a paper reporting the effects of rhGH on mixed lymphocyte cultures from renal allograft recipients against donor tissue. Out of the 12 patients tested, 3 patients had augmented responses when rhGH was added to the culture; these 3 patients had biopsy proven chronic rejection (Benfield *et al.* 1996). If rhGH does stimulate the immune system, then an increase in activation markers would be expected. No such increase was seen in any of the three T cell activation markers tested in our study. Indeed it was not possible to distinguish those patients who went on to develop rejection episodes from those who did not. Expression of activation markers as tested in our study does not predict who will reject during rhGH treatment and who will not.

The rejection rate within our transplanted group is high compared to published data. There are a number of explanations for this. The episodes were presumed rejection, as not all children underwent renal biopsy, and so the incidence is likely to be higher than in centres where all suspected rejection is investigated by renal biopsy. However the same diagnostic criteria for rejection were used for both phases of the trial. Secondly, our patients are selected in that we have studied only slowly growing children, some of whom had poor graft function with biopsy evidence of chronic rejection before receiving rhGH treatment. Thirdly, two children accounted for six of the nine episodes.

Growth hormone treatment has been reported to increase haemoglobin concentration in children with short stature due to various conditions (Vihervuori *et al.* 1994). In these studies, mean haemoglobin had increased by 0.7 g/dl after 6 months ($p < 0.001$), and over 50% of the children had evidence of iron deficiency, presumed to be due to depletion of iron stores. Insulin-like growth factor-I enhances the actions of erythropoietin and indeed has erythropoietin-like activity at high doses (Golde *et al.*

1977; Kurtz *et al.* 1988; Aron *et al.* 1992). The authors also suggest that renal growth during rhGH therapy could result in increased production of erythropoietin. In a previous report we were unable to demonstrate any change in the volume of transplanted kidneys during rhGH treatment (Maxwell *et al.* 1996b). We were unable to demonstrate an increase in haemoglobin in either of our groups of patients. None received erythropoietin before or during the study. Many of our patients had evidence of anaemia of renal failure at the start of the study. In our patients, lack of endogenous erythropoietin may be the limiting factor preventing a rise in haemoglobin during rhGH treatment.

Conclusion

Growth hormone treatment resulted in a slight but significant reduction in CD4⁺ cells in the CRI group, but there were no significant changes in lymphocyte subsets in the transplanted group. There were no consistent changes in T lymphocyte activation markers in either group. There was no change in the incidence of infection, or rejection episodes in the transplanted group. It was not possible to distinguish those children with rejection episodes from those without, either by lymphocyte subset or by activation markers. There was no increase in haemoglobin concentration during rhGH treatment.

Table 9.1 Patient details in the CRI and transplanted groups

	CRI	Renal Transplant
Number Boys / Girls	11 / 2	7 / 3
Age	8.8 (4.9 - 13.9)	12.3 (9.4 - 15.2)
GFR	22 (9 - 59)	61 (26 - 93)
Ht SDS	-3.2 (-4.8 to -2.2)	-2.7 (-3.5 - -1.6)
HV	4.4 (1.7 - 6.5)	4.2 (2.6 - 5.6)
Prepubertal / Pubertal		
Aetiology of renal Disease		
Congenital Uropathy	10	6
FSGS	3	-
Congenital Nephrotic Syndrome	-	1
Cystinosis	-	1
HUS	-	1
Nephronophthisis	-	-
Unknown	-	1

Table 9.2a Haemoglobin, lymphocyte, neutrophil and platelet counts during rhGH treatment in the CRI group

	Day 1	Day 8	3 Mths	6 Mths	1 Year
	<i>n=13</i>	<i>n=13</i>	<i>n=13</i>	<i>n=13</i>	<i>n=11</i>
Haemoglobin g/dl	10.6 (8.7-13.3)	9.9 (7.3-13.3)	10.4 (8.8-13.3)	10.3(7.8-13.3)	9.5 (7.5-12.7)
Lymphocytes x 10⁹/l	2.9 (1.5-4.7)	3.0 (1.5-4.9)	2.8 (0.8-5.9)	2.7 (1.7-4.2)	2.6 (1.5-4.9)
Neutrophils x 10⁹/l	3.1 (1.5-6.4)	3.2 (1.4-6.4)	4.1 (1.1-8.6)	3.2 (2.3-4.9)	3.3 (0.8-7.4)
Platelets x 10⁹/l	322(155-739)	288(155-427)	314(136-523)	281(143-383)	272(127-380)

Table 9.2b Haemoglobin, lymphocyte, neutrophil and platelet counts during rhGH treatment in the transplanted group

	Day 1	Day 8	3 Mths	6 Mths	1 Year
	<i>n=10</i>	<i>n=10</i>	<i>n=10</i>	<i>n=10</i>	<i>n=8</i>
Haemoglobin g/dl	11.3 (7.4-13.8)	10.6 (6.3-13.3)	11.2 (6.5-14.9)	10.9(6.9-14.4)	11.1 (6.2-13.7)
Lymphocytes x 10⁹/l	1.8 (0.6-3.9)	1.8 (0.4-3.1)	1.8 (0.3-4.2)	1.6 (0.6-3.2)	1.9 (0.4-3.2)
Neutrophils x 10⁹/l	4.1 (2.3-6.7)	5.4 (2.0-12.6)	5.1 (1.6-8.9)	4.1 (2.1-6.5)	4.5 (2.4-6.2)
Platelets x 10⁹/l	294(185-376)	286(155-409)	301(235-436)	305(247-382)	271(207-309)

Table 9.3 Mean (SE) total lymphocyte count, lymphocyte subsets and % activated T cells during the control and treatment phases in the transplanted group

	<u>Control Phase</u> <i>n</i> = 7	<u>Treatment Phase</u> <i>n</i> =10	
Total Lymphocytes	1.88 (0.35)	1.82 (0.34)	<i>p</i> = 0.78
T Cells	1.40 (0.26)	1.38 (0.29)	<i>p</i> = 0.92
CD4⁺ Cells	0.80 (0.18)	0.80 (0.17)	<i>p</i> = 0.99
CD8⁺ Cells	0.52 (0.12)	0.50 (0.15)	<i>p</i> = 0.82
B Cells	0.11 (0.02)	0.07 (0.02)	<i>p</i> = 0.015
NK Cells	0.25 (0.14)	0.11 (0.02)	<i>p</i> = 0.33
<u>% T Cells Expressing</u>			
CD25	15 (3)	7 (1)	<i>p</i> = 0.013
CD26	38 (7)	34 (5)	<i>p</i> = 0.65
HLA-DR	25 (7)	16 (5)	<i>p</i> = 0.34

Table 9.4 Mean (SE) change in haemoglobin, total WCC, lymphocyte, neutrophil and platelet counts, lymphocyte subsets and % activated T cells during the control and treatment phases in the transplanted group

	<u>Control Phase</u> <i>n</i> = 7	<u>Treatment Phase</u> <i>n</i> =9	
Haemoglobin (g/dl)	-0.5 (0.1)	-0.3 (0.2)	<i>p</i> = 0.57
Total WCC x 10 ⁹ /l	0.9 (0.7)	-0.7 (0.6)	<i>p</i> = 0.16
Lymphocytes x 10 ⁹ /l	0.0 (0.2)	0.0 (0.3)	<i>p</i> = 0.89
neutrophils x 10 ⁹ /l	0.4 (0.9)	0.2 (0.6)	<i>p</i> = 0.87
Platelets x 10 ⁹ /l	-7 (13)	-34 (21)	<i>p</i> = 0.39
B Cells	-0.04 (0.02)	-0.03 (0.02)	<i>p</i> = 0.72
NK Cells	0.03 (0.02)	-0.19 (0.14)	<i>p</i> = 0.24
CD3	-0.19 (0.1.)	0.06 (0.24)	<i>p</i> = 0.42
CD4	-0.08 (0.05)	4.14 (0.21)	<i>p</i> = 0.69
CD8	-0.14 (0.12)	-0.01 (0.10)	<i>p</i> = 0.45
<u>% T cells expressing</u>			
CD25	-3 (2)	-9 (4)	<i>p</i> = 0.31
CD26	-4 (5)	-0.8 (12)	<i>p</i> = 0.56
HLA-DR	-14 (13)	15 (7)	<i>p</i> = 0.94

Table 9.5a Mean lymphocyte count and lymphocyte subsets during rhGH treatment in the CRI group

	Normal Range	Day 1	3 Mths	6 Mths	1 Year
LC x 10⁹/l	1.5 - 7.0	2.9 ± 0.9	2.8 ± 1.3	2.7 ± 0.7	2.6 ± 1.0
CD3⁺ x 10⁹/l	1.82 ± 0.48	2.16 ± 0.70	1.91 ± 0.62*	1.88 ± 0.57*	1.53 ± 0.70*
CD4⁺ x 10⁹/l	0.98 ± 0.24	1.33 ± 0.47	1.01 ± 0.55*	1.19 ± 0.52*	0.97 ± 0.66**
CD8⁺ x 10⁹/l	0.72 ± 0.22	0.66 ± 0.32	0.47 ± 0.33	0.60 ± 0.27	0.48 ± 0.23
B Cells x 10⁹/l	0.05 - 0.50	0.30 ± 0.10	0.33 ± 0.22	0.27 ± 0.19	0.34 ± 0.21
NK Cells x 10⁹/l	0.08 - 0.65	0.31 ± 0.22	0.80 ± 0.87	0.44 ± 0.29	0.53 ± 0.46

* ANOVA $p = 0.03$, ** ANOVA $p = 0.04$

Table 9.5b Mean lymphocyte count and lymphocyte subsets during rhGH treatment in the transplanted group

	Normal Range	Day 1	3 Mths	6 Mths	1 Year
LC x 10⁹/l	1.5 - 7.0	1.8 ± 1.2	1.8 ± 1.3	1.6 ± 1.0	1.9 ± 1.2
CD3⁺ x 10⁹/l	1.82 ± 0.48	1.40 ± 0.93	1.48 ± 1.14	0.99 ± 0.58	1.64 ± 1.04
CD4⁺ x 10⁹/l	0.98 ± 0.24	0.75 ± 0.64	0.86 ± 0.66	0.59 ± 0.40	0.88 ± 0.61
CD8⁺ x 10⁹/l	0.72 ± 0.22	0.49 ± 0.43	0.56 ± 0.27	0.23 ± 0.18	0.52 ± 0.44
B Cells x 10⁹/l	0.05 - 0.50	0.10 ± 0.08	0.06 ± 0.07	0.08 ± 0.12	0.07 ± 0.07***
NK Cells x 10⁹/l	0.08 - 0.65	0.21 ± 0.48	0.08 ± 0.06	0.19 ± 0.20	0.08 ± 0.09

*** ANOVA $p = 0.004$

Table 9.6a Mean (SD) % activated T cells during rhGH treatment in the CRI group

	Day 1	3 Mths	6 Mths	1 Year
CD25	11.7 (11.8)	10.5 (5.5)	9.3 (3.9)	6.7 (4.8)
CD26	23.5 (8.1)	41.6 (18.1)	39.8 (15.8)	28.3 (22.5) *
DR	19.8 (21.6)	19.4 (11.0)	20.9 (20.8)	11.1 (5.4)

* increased expression of CD26 (ANOVA $p=0.03$)

Table 9.6b Mean (SD) % activated T cells during rhGH treatment in the transplanted group

	Day 1	3 Mths	6 Mths	1 Year
CD25	14.8 (11.6)	8.8 (8.6)	6.0 (5.2)	6.2 (5.3)
CD26	39.4 (24.5)	27.4 (17.6)	43.0 (21.3)	37.0 (22.3)
DR	27.4 (22.8)	12.2 (7.9)	8.3 (5.7)	9.0 (5.0)

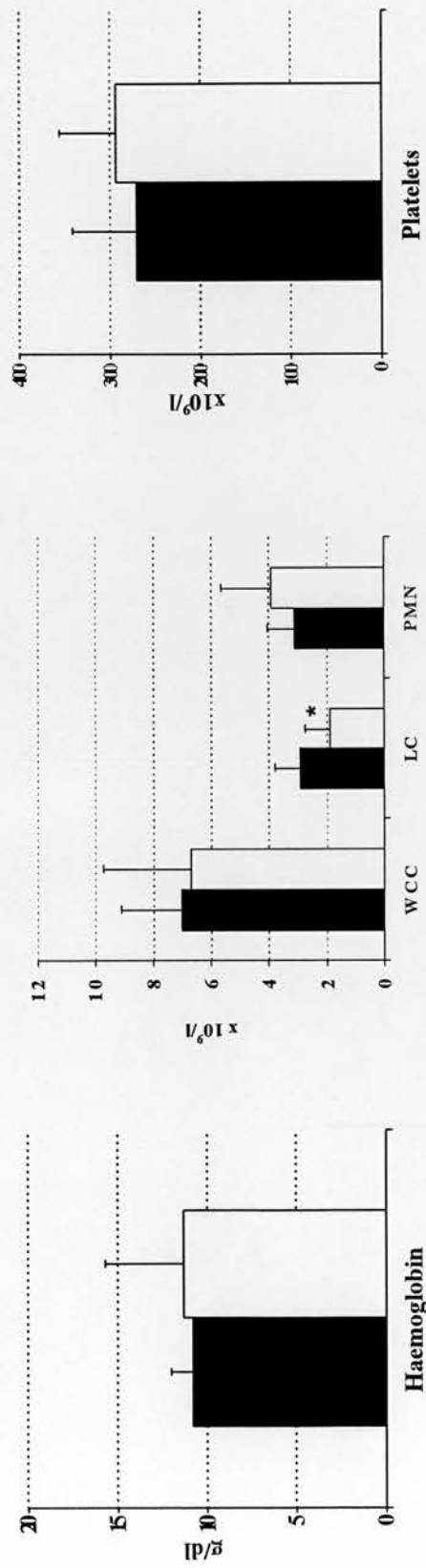


Figure 9.1 Mean (SD) haemoglobin, total white cell (WCC), lymphocyte (LC), neutrophil (PMN) and platelet counts at the start of treatment in the CRI and transplanted groups. The lymphocyte count was lower in the transplanted group compared to the CRI group, * $p = 0.049$.

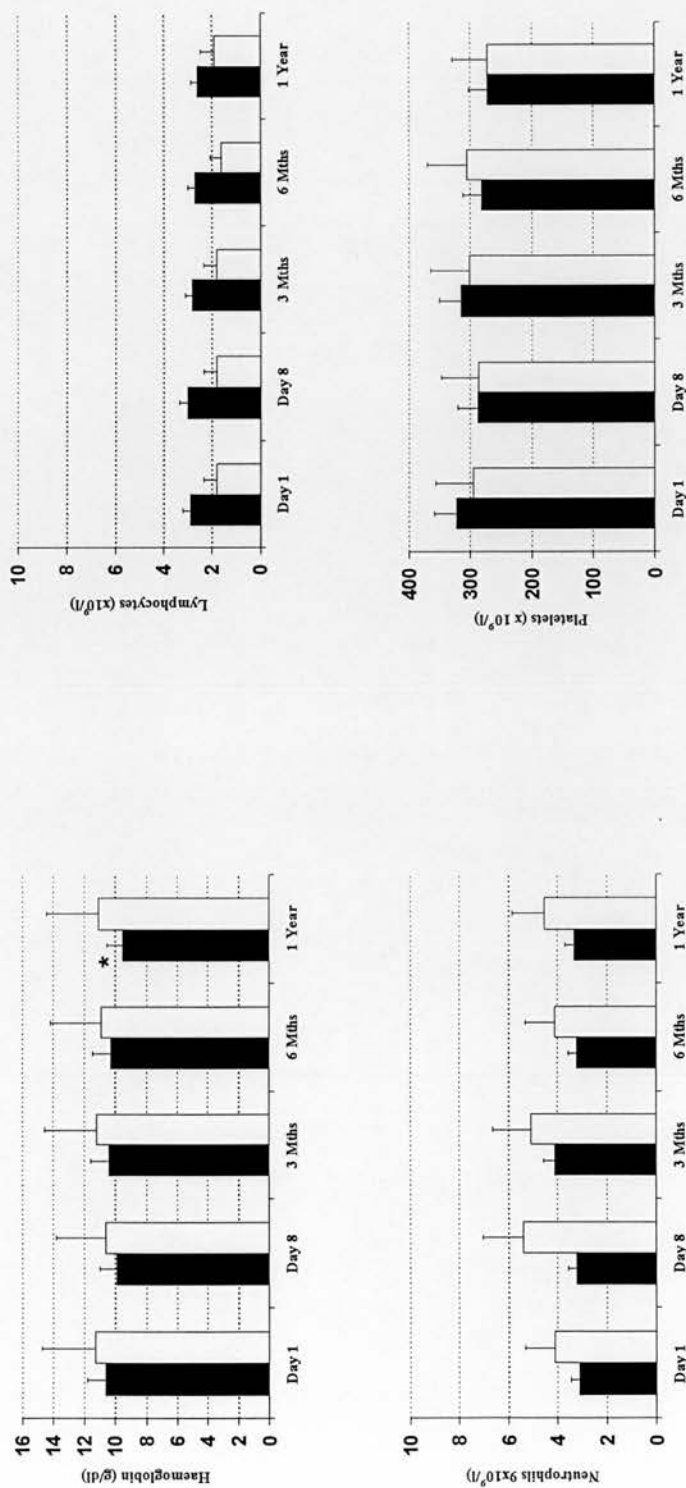


Figure 9.2 Mean (SD) haemoglobin, lymphocyte, neutrophil and platelet counts during rhGH treatment in the CRI ■ and transplanted groups □. Haemoglobin decreased during the study in the CRI group, * $p = 0.007$ ANOVA.

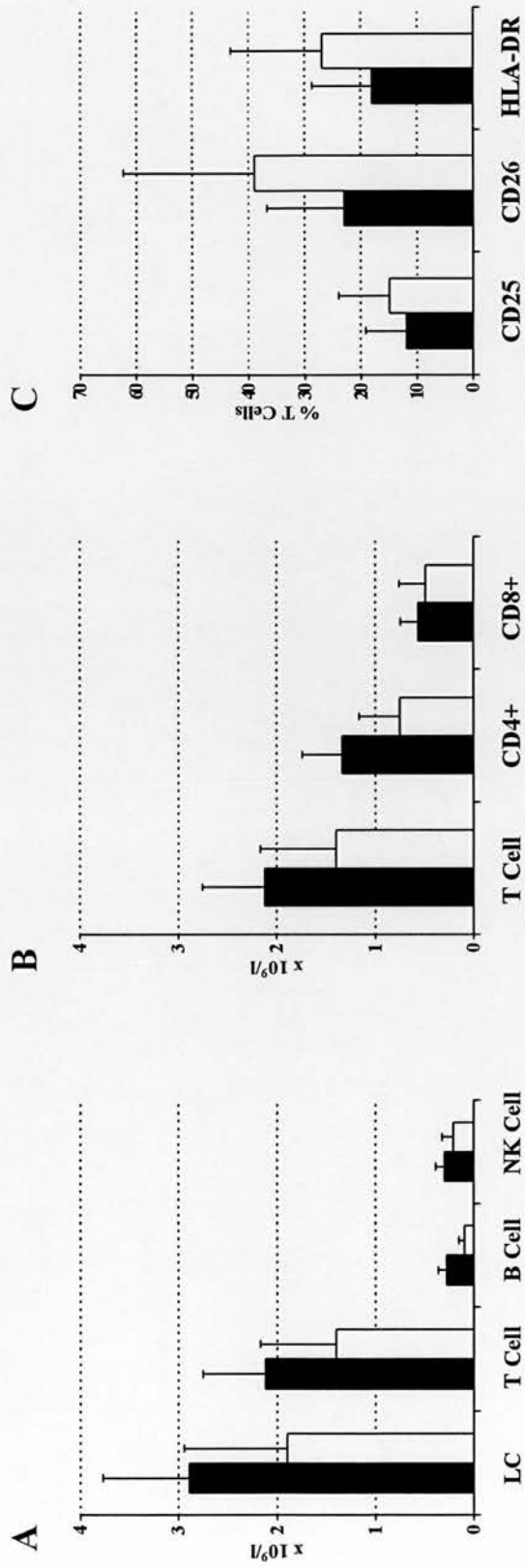


Figure 9.3 Mean (SD) values of each variable at baseline in the CRJ and transplanted groups \square for (A) total lymphocytes, T cells (CD3), B cells and natural killer (NK) cells; (B) T cells, CD4+ and CD8+ cells; (C) % T cells positive for CD25, CD26 and HLA-DR.

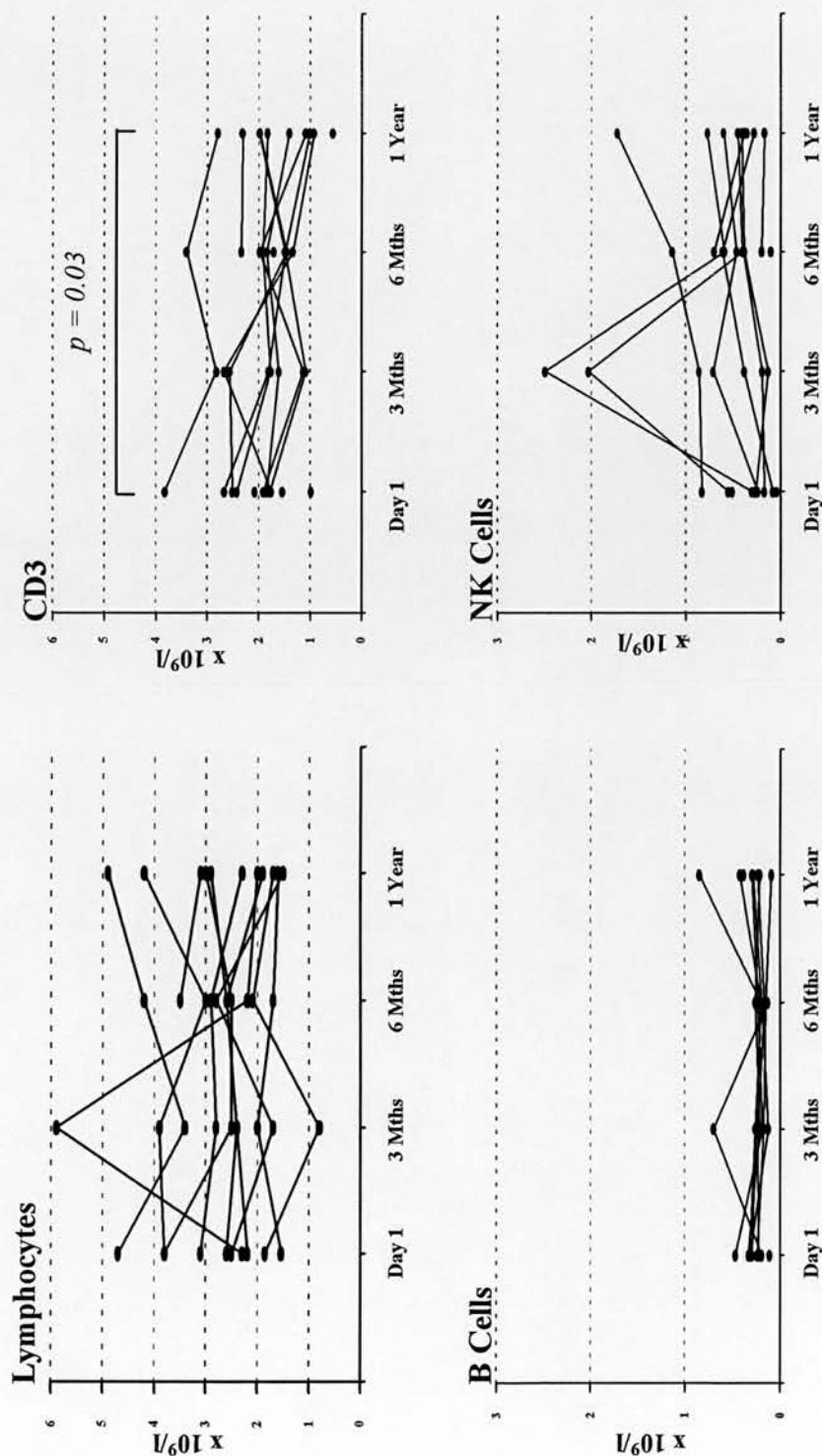


Figure 9.4 Total lymphocytes, T cells (CD3), B cells and natural killer (NK) cells during rhGH treatment in the CRI group. Note that the scale is larger in the lower two graphs. The CD3 counts decreased during treatment, $p = 0.03$ ANOVA.

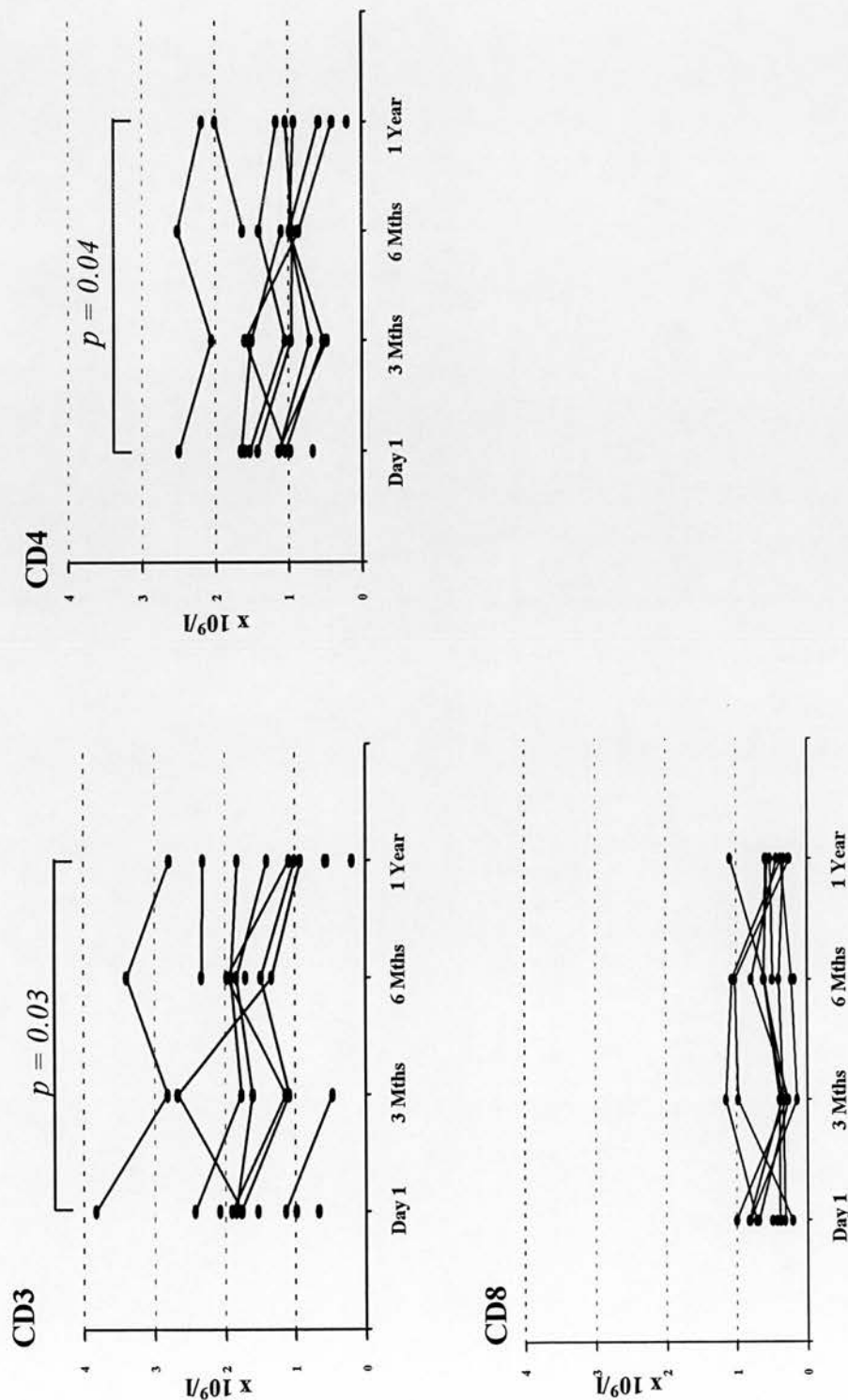


Figure 9.5 T cells (CD3), and CD4+ and CD8+ T cells during rhGH treatment in the CRI group. The CD3 count decreased during treatment, $p = 0.03$, ANOVA, as did the CD4 count, $p = 0.04$, ANOVA.

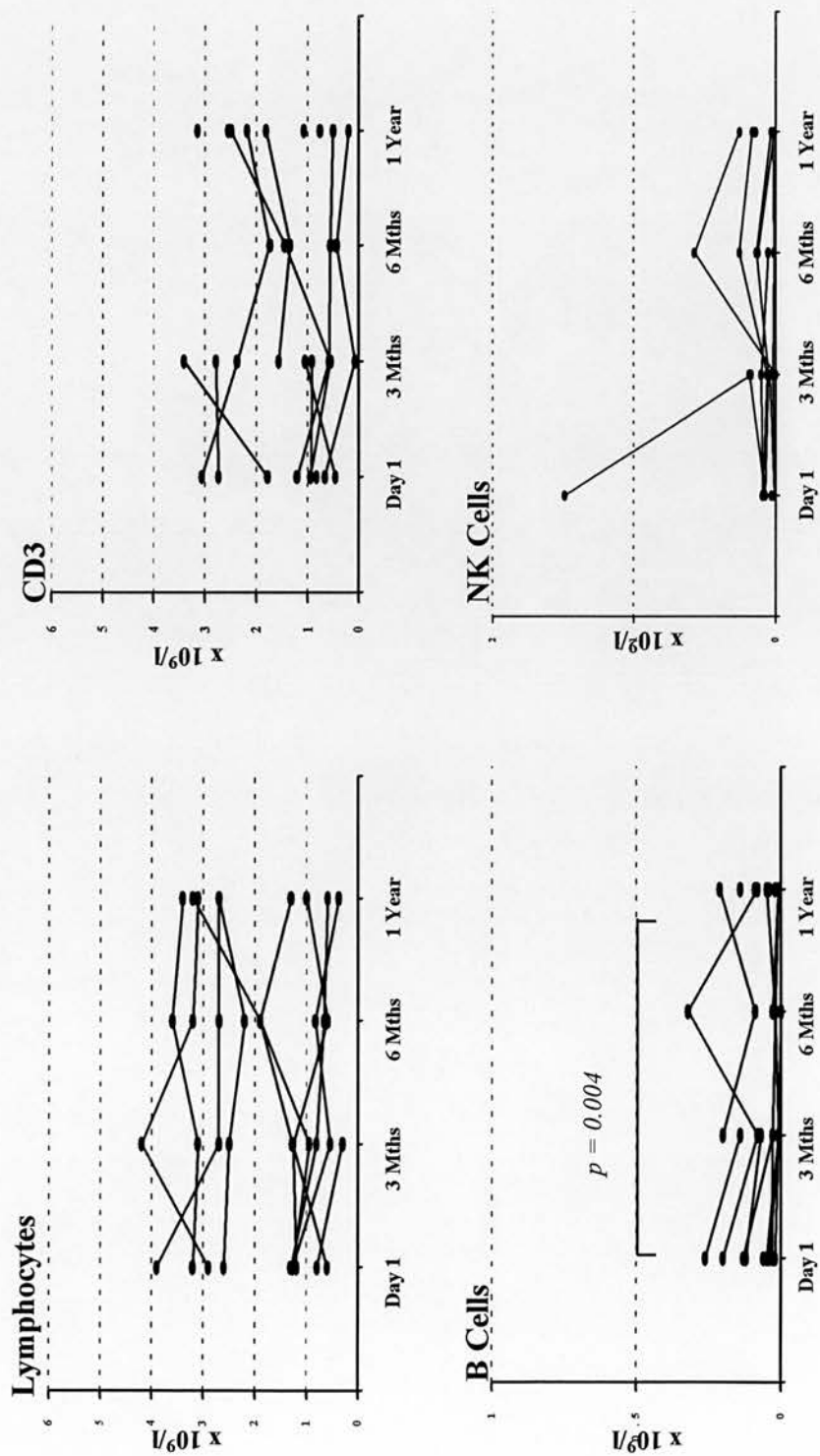


Figure 9.6 Total lymphocytes, T cells (CD3), B cells and natural killer (NK) cells during rhGH treatment in the transplanted group. Note the different scales in the lower two graphs. The number of B cells decreased during the study, $p = 0.004$, ANOVA.

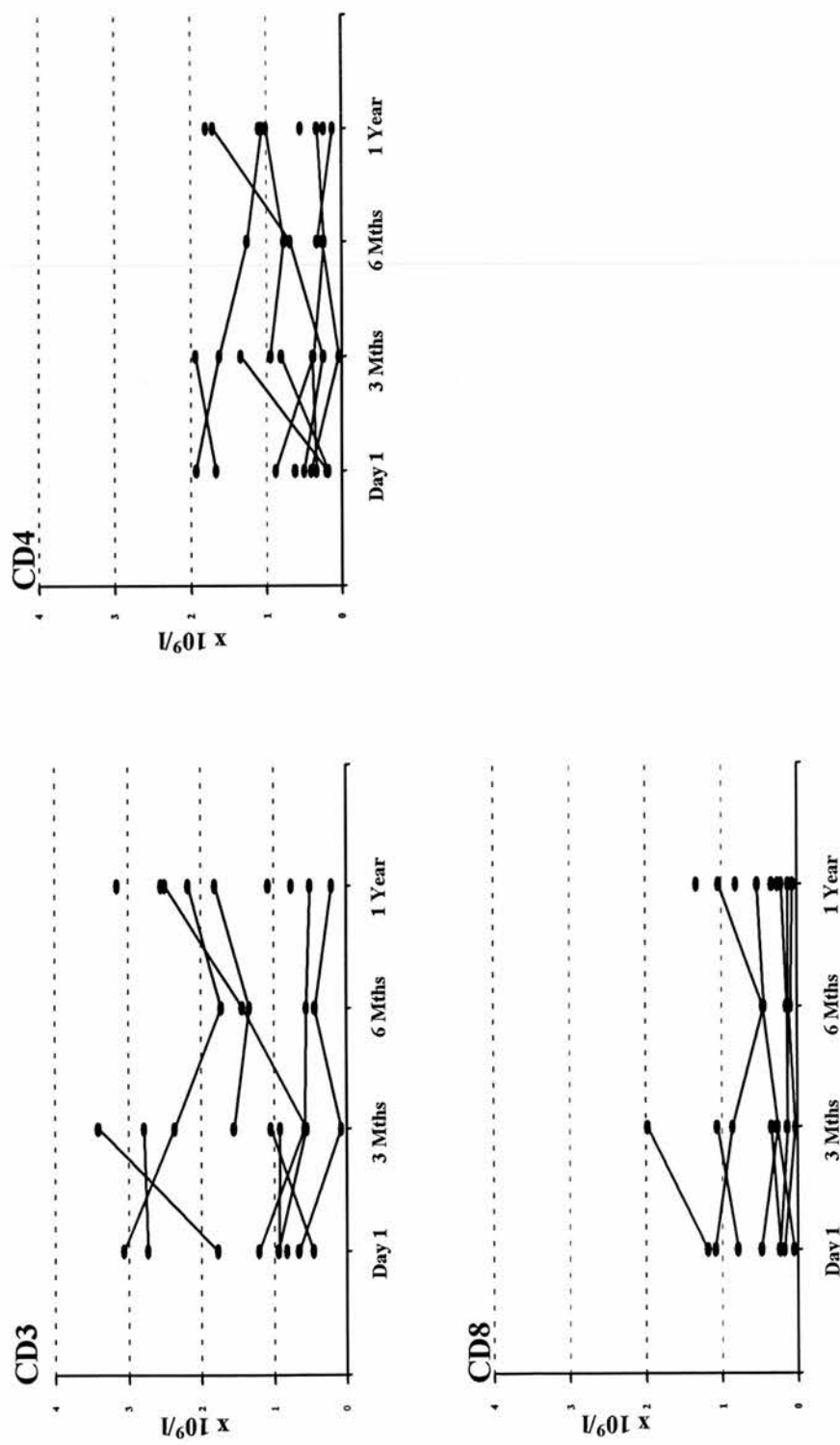


Figure 9.7 T cells (CD3), and CD4+ and CD8+ T cells during rhGH treatment in the transplanted group.

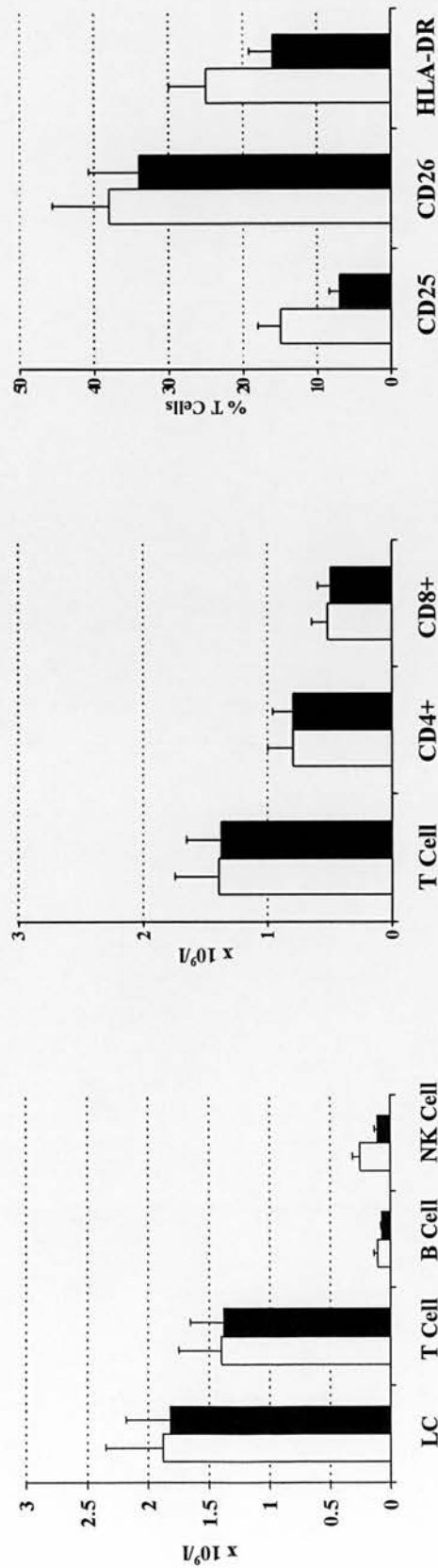


Figure 9.8 Mean (SE) of the averaged values of each variable during the control \square and treatment phases \blacksquare are shown for: (A) total lymphocytes, T cells (CD3), B cells and natural killer (NK) cells; (B) T cells, CD4+ and CD8+ cells; (C) % T cells positive for the activation markers CD25, CD26 and HLA-DR.

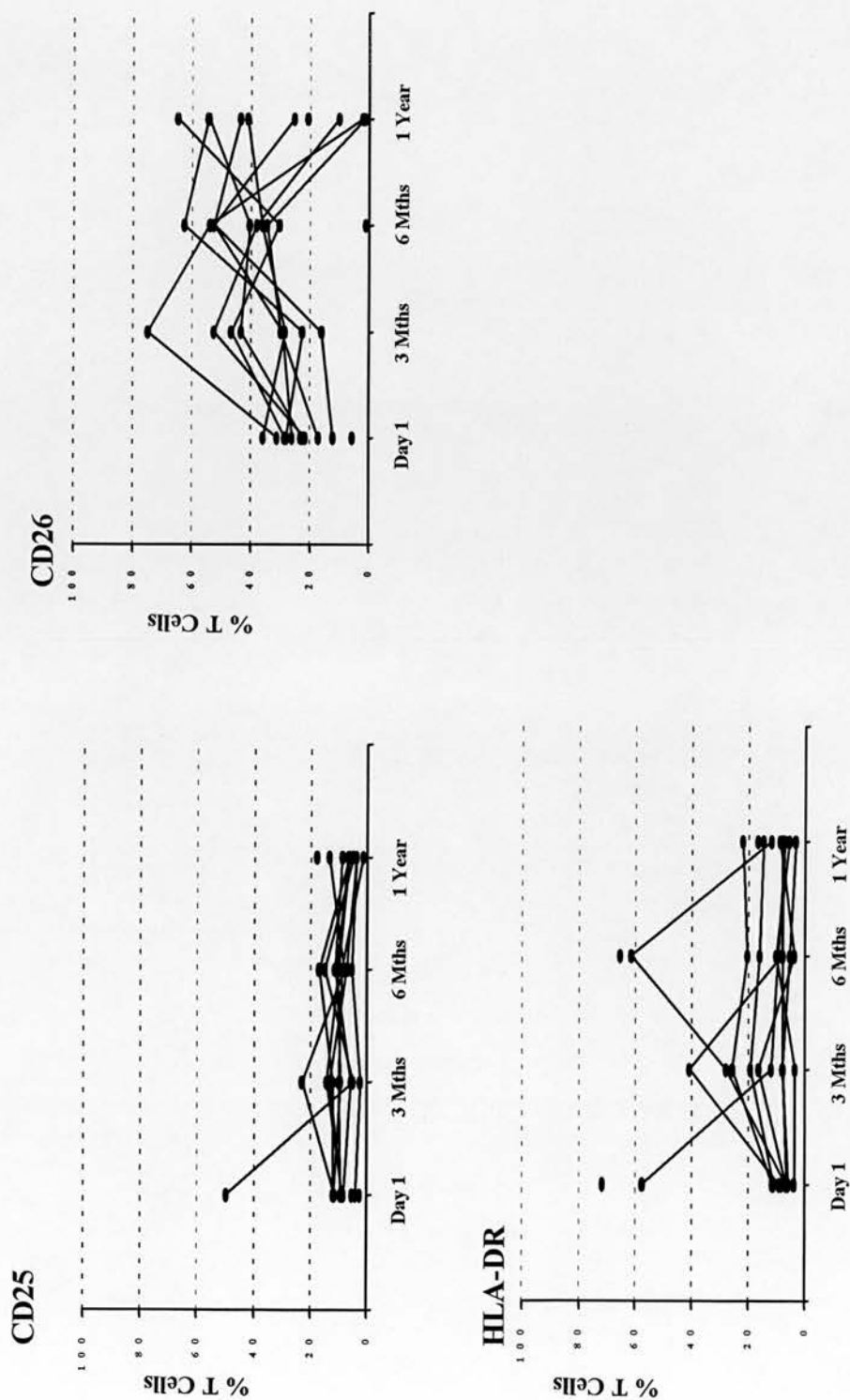


Figure 9.9 Percentage T cells expressing CD25, CD26 and HLA-DR during rhGH treatment in the CRI group.

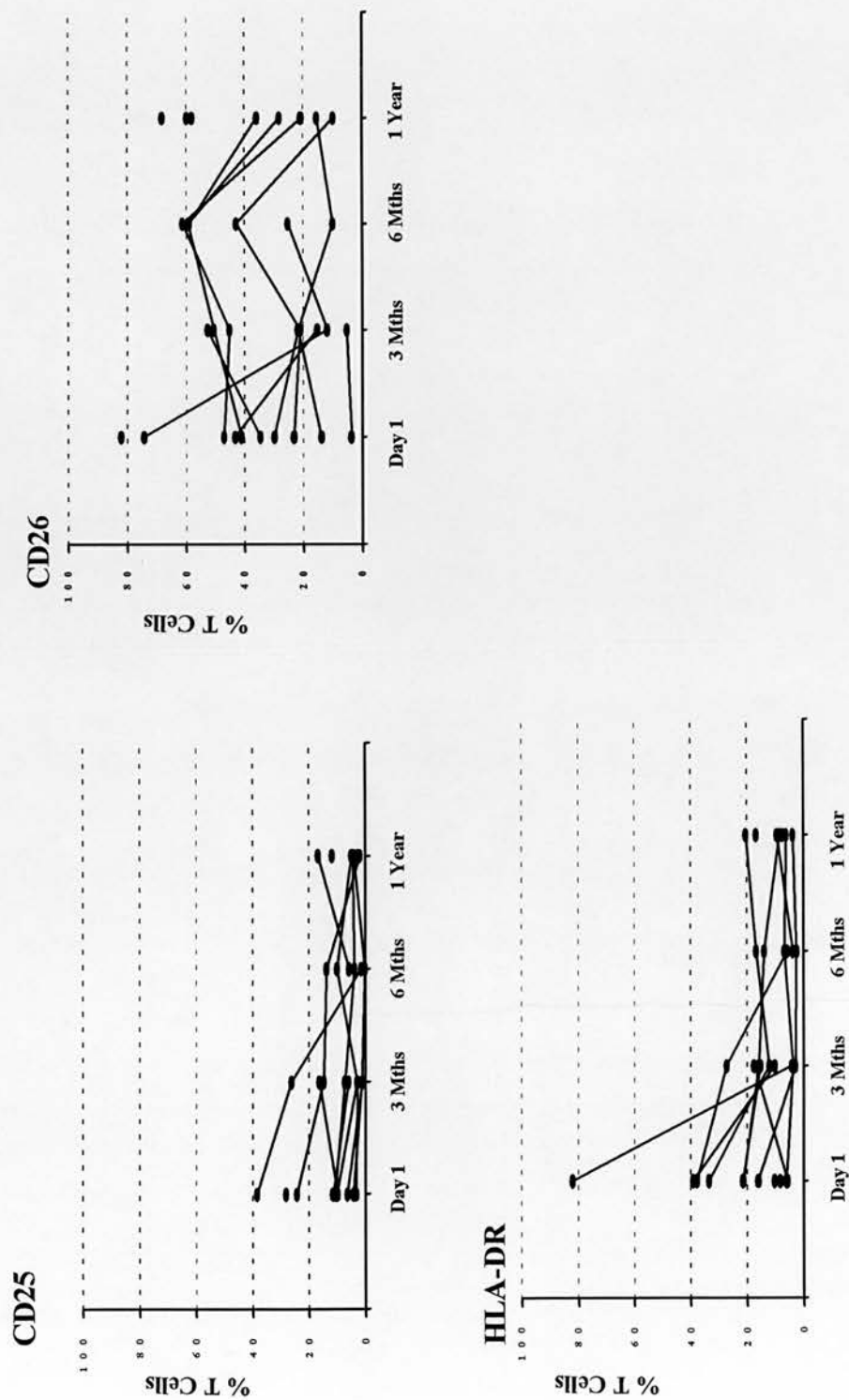


Figure 9.10 Percentage T cells expressing CD25, CD26 and HLA-DR during rhGH treatment in the transplanted group.

Chapter 10: The Metabolic Effects Of Recombinant Human Growth Hormone In Children With Chronic Renal Insufficiency and Renal Transplants

Introduction

Growth hormone has a role in carbohydrate and lipid metabolism, and its use in supraphysiological doses in CRF requires carefully monitoring. Patients with CRF already have glucose intolerance and abnormal lipid profiles which might be exacerbated by rhGH treatment. Growth hormone not only stimulates growth but also affects bone mineral content. This may be relevant in children with CRF who will have varying degrees of renal osteodystrophy. This chapter details the effects of rhGH on glucose metabolism, lipid profiles and on parameters of bone metabolism in each of the different arms of the trial.

Methods

Different protocols were used for each of the arms of the BAPN study, so the data collected varied between the groups. Details are given in the appropriate section below. Children attending the London centres, were seen on day 1, day 8 and then three monthly. All other children were seen at three monthly intervals for one year (infants, CRI and dialysis groups) or two years (transplanted children). Within groups the results are expressed as the mean (SD) at each time point; comparison of groups are expressed as the mean (SE). Where the distribution of data points was skewed, the median valued are reported. Analysis of variance was used to assess changes within each group during treatment. Multiple regression analysis was used to perform correlations within groups.

Patients

Patient details for each group have already been described in Chapter 3 (infants), Chapter 4 (CRI), Chapter 5 (dialysis), and Chapter 6 (transplanted patients). The

results for glucose metabolism, lipids and renal bone disease are reported and discussed under separate headings below.

I. GLUCOSE METABOLISM

Glucose Metabolism in Chronic Renal Failure

Abnormal glucose metabolism is a common finding in patients with CRF (DeFronzo *et al.* 1978; Alvestrand 1997). Several mechanisms contribute, but the most important is peripheral resistance to the actions of insulin (DeFronzo *et al.* 1981). The major site of insulin resistance is skeletal muscle, where a post-receptor defect has been described (Smith and DeFronzo 1982). Dialysis enhances, but does not normalise, insulin-mediated glucose disposal (DeFronzo *et al.* 1978). Similarly correction of anaemia in CRF, by the use of erythropoietin, improves glucose disposal (Mak 1996), as does correction of metabolic acidosis (Reaich *et al.* 1993). In patients with CRF, insulin-mediated suppression of hepatic gluconeogenesis is reduced (Schmitz 1985).

In many patients the effect of insulin resistance is offset by increased insulin secretion by the pancreatic β cells (Mak 1985), but if there is no increased insulin secretion, then overt glucose intolerance occurs. Hyperparathyroidism is known to reduce insulin secretion by the pancreas in uraemia; parathyroidectomy for secondary hyperparathyroidism increases insulin secretion (Mak 1985).

The kidneys are involved in the metabolic clearance of insulin, which is reduced in renal failure (DeFronzo *et al.* 1978). Haemodialysis improves the metabolic clearance of insulin; this is thought to be due to an increase in the peripheral clearance of insulin (Gin *et al.* 1994).

Other factors also affect glucose tolerance in patients with renal failure; renal transplant patients receiving steroids have a further risk factor for glucose intolerance

(Fennel *et al.* 1983), and glucose tolerance during childhood is also described as being affected by age and by obesity (Deschamps *et al.* 1977; Mak *et al.* 1983; Amiel *et al.* 1986).

Growth Hormone and Glucose Metabolism

It is well recognised that patients with acromegaly and those with GH-secreting tumours can develop glucose intolerance and overt diabetes (Sonksen *et al.* 1967; Rizza *et al.* 1982). The effects of GH on glucose metabolism can also be inferred both from the study of patients with GHD, and by looking at the effects of GH administration in normal subjects. Children with complete GHD develop hypoglycaemia when fasted (Hopwood *et al.* 1975). This is due to reduced hepatic gluconeogenesis and possibly also reduced glycogenolysis (Haymond *et al.* 1976), possibly mediated by an increase in the net effect of insulin in a setting of reduced GH antagonism. In GHD, insulin secretion is reduced (Underwood *et al.* 1973).

In an experimental setting, administration of large doses of intravenous GH to normal subjects results in inhibition of peripheral glucose uptake, but no increase in hepatic glucose production (Rizza *et al.* 1982, Rabinowitz *et al.* 1965). Patients with acromegaly have both reduced peripheral uptake and increased hepatic synthesis of glucose (Karlander *et al.* 1986). The effects of GH are however countered by an increase in insulin secretion which occurs with either GH excess or GH administration (Salomon *et al.* 1989). A diabetogenic effect of GH is only seen if this compensatory mechanism fails.

In childhood GHD, GH replacement has been shown to restore glucose production to normal and to increase insulin secretion (Underwood *et al.* 1973). Growth hormone treatment of short normal children does not change glucose turnover nor does it affect glucose tolerance and sensitivity to insulin remains intact (Walker *et al.* 1989). There was however an increase in insulin secretion for the duration of treatment (Walker *et al.* 1989). Similar results have been reported with GH replacement in adults with GHD (Salomon *et al.* 1990).

Chronic renal failure patients are already at risk of glucose intolerance, and it is therefore important to determine whether rhGH treatment can be tolerated or whether it further aggravates the situation.

Methods

Fasting glucose and HbA1c levels were measured in all groups of patients. Fasting insulin was available only in the infant, CRI and transplanted groups. The insulin data were skewed and therefore the median rather than the mean values are reported.

Results

Glucose

At the start of the study, there were several children in each subgroup who had fasting glucose levels above the normal range, as shown in Figure 10.1. There were no differences between the groups at baseline, and no significant changes in fasting glucose during the first year of rhGH treatment in any group (Table 10.1, Figures 10.2 and 10.3). Within the transplanted group there was no difference between the control and treatment groups during the first year of study (Figure 10.4). In the CRI group, fasting glucose level prior to treatment was best predicted by age ($r^2 = 0.134$, $p = 0.028$).

Insulin

At the start of the study, there was a trend for higher fasting insulin values in the transplanted group, where several children had values outwith the normal range, compared with the infant group where only 2 children had values above the expected range ($p = 0.07$), and the CRI group, where only one child had a value above normal ($p = 0.08$), Table 10.1, Figure 10.5. Median values for fasting insulin are given. In the CRI group at baseline, fasting insulin was correlated with age ($p = 0.043$) and height SDS ($p = 0.011$), Figure 10.6. There was no relation to GFR, WFH or PTH. Fasting

insulin was unrelated to the variables tested in the transplant group (prednisolone dose, GFR, WFH, HVSDS, height SDS).

During rhGH treatment, fasting insulin values were elevated at 1 week in both the transplanted ($p = 0.02$) and CRI groups ($p = 0.01$), but returned toward baseline during the first year of rhGH treatment, Table 10.1, Figure 10.7. Insulin values in the transplanted control and treatment groups are shown in Figure 10.8. There was considerable variation, but an increase was seen after 1 week ($p = 0.03$), 3 months ($p = 0.04$), and 6 months ($p = 0.05$) in the treatment group, whilst there was no change in the control group at any of these time points.

HbA1c

HbA1c at the start of treatment is shown in Figure 10.9. Mean HbA1c in the transplanted group was higher than in the CRI group ($p = 0.003$), and the combined dialysis group ($p < 0.0001$). Using multiple regression analysis, HbA1c at baseline in the CRI group, was directly correlated with weight for height ($p = 0.006$) and inversely correlated to HVSDS ($p = 0.025$). There was no relation to age, GFR nor PTH. In the transplanted group, HbA1c was inversely correlated with HVSDS ($p = 0.003$). There was no correlation with prednisolone dose, age, GFR, WFH nor PTH. There was no significant change in HbA1c during treatment in any of the groups.

Discussion

Chronic Renal Insufficiency

In keeping with the known occurrence of glucose intolerance in uraemia, 4 out of 27 children in the CRI group had an elevated fasting glucose above the normal range at baseline. These children were indistinguishable from the rest of the group in terms of renal function, but within the group, fasting glucose was strongly correlated with age. Insulin sensitivity in normal children has previously been shown to be influenced by age (Amiel *et al.* 1986), with older children showing greater degrees of glucose

intolerance. Other authors have also found glucose tolerance to be related to age in children with CRF (Mak *et al.* 1983; Haffner *et al.* 1998b).

In the CRI group, fasting insulin at baseline was positively correlated with height SDS, and in both the CRI and transplanted groups HbA1c was inversely correlated with HVSDS. Assuming that HbA1c is a marker of the degree of insulin resistance or insulin lack, these results are consistent with the known anabolic effects of insulin.

Haffner reported the results of oral glucose tolerance tests (OGTT) in children with CRF before and after rhGH. Prior to starting rhGH treatment, 3 out of 29 patients on conservative management had an impaired OGTT, and in accordance with our results, there was no change in fasting glucose or OGTT during rhGH treatment for up to 5 years (Haffner *et al.* 1998b). Saenger also reported no change in glucose tolerance during 5 years of rhGH treatment in CRI (Saenger *et al.* 1996).

In the CRI group, fasting insulin levels were also mildly elevated. There was a small but significant rise in fasting insulin during rhGH treatment. Haffner studied CRI patients receiving rhGH for up to 5 years; fasting insulin at baseline was higher than control values, and rose during rhGH treatment, but this only became significantly different after 3 years of treatment (Haffner *et al.* 1998b). Fine, reporting a two year placebo controlled study of rhGH in CRI, found no change in fasting glucose with rhGH compared to placebo, but fasting insulin increased in the rhGH treatment group compared to the control group (Fine *et al.* 1994). Continued follow up of these patients found that after 5 years of rhGH treatment insulin remained significantly increased compared to baseline, and HbA1c increased from 5.3% at baseline to 6.1% ($p = 0.003$) (Fine *et al.* 1996b). This is in contrast to the CRI data reported by Haffner, where there was no change in HbA1c after 5 years of rhGH treatment (Haffner *et al.* 1998b).

There were no changes in HbA1c in our patients, and Van Es reported no change in HbA1c during 2 years of rhGH treatment in 31 patients with CRI (Van Es *et al.*

1991). It is likely that the differences between these studies reflect individual patient variation in terms of GFR, body mass, nutrition, age and other factors.

The glucose and insulin data in the CRI patients are consistent with rhGH treatment increasing insulin secretion, but leaving glucose turnover unchanged. Whilst it may be reassuring that rhGH does not result in insulin resistance, prolonged hyperinsulinaemia could turn out to be an unwanted longterm cardiovascular risk factor for these children.

Dialysis

At baseline, fasting glucose in the dialysis arm of the study was no different to the CRI or transplanted groups, and there was no change in fasting glucose, nor of HbA1c during rhGH treatment. Of potential concern during peritoneal dialysis, children absorb approximately 2-3 g/kg/day of glucose from the peritoneal fluid, and the amount is even greater when hypertonic dialysate is used to increase fluid removal. However glucose tolerance appears to be no worse in patients receiving peritoneal dialysis compared to haemodialysis, and has even been reported to improve following the institution of peritoneal dialysis (Heaton *et al.* 1989). Perhaps surprisingly HbA1c levels at the start of treatment were higher in the transplanted group than in the combined dialysis groups; a finding also reported by Haffner (Haffner *et al.* 1998b). Steroids would appear to have more of a detrimental effect on glucose intolerance than uraemia, however data regarding the metabolic effects of rhGH in dialysis patients are more limited than for other groups of CRF patients, as most paediatric dialysis patients only remain on dialysis for a short period of time before receiving a renal transplant.

Renal Transplantation

Four of 21 transplanted children also had an elevated fasting glucose at baseline, but there was no change in mean fasting glucose during rhGH treatment. Before treatment, there was a tendency for fasting insulin to be higher in this group compared with the CRI group, and whilst there was an increase in fasting insulin during treatment, this was not sustained.

It is well established that glucose tolerance is abnormal in children with renal transplants. Fennell was one of the first authors to report that glucose tolerance was adversely affected by steroid treatment (Fennell *et al.* 1983). In 1991, Van Dop reported the results of OGTT in 15 children with renal transplants; 3 of these children had abnormal OGTT results. During the OGTT, the integrated glucose concentration was directly correlated with adiposity and indirectly correlated with prednisolone dose, whilst the integrated insulin concentration was directly correlated with prednisolone dose and age. Integrated glucose and insulin concentrations were unaltered by 12 months of rhGH treatment in 10 of these children (Van Dop *et al.* 1991). In our patients neither fasting glucose nor insulin levels were related to prednisolone dosage.

Likewise Guest has reported similar findings (Guest *et al.* 1998). Of 49 children with renal transplants who were tested, 44 had normal glucose tolerance and 5 had impaired OGTT. Eleven children had evidence of insulin resistance. In this study fasting glucose was proportional to fasting insulin ($p = 0.04$). These children were then entered into a controlled trial of rhGH treatment. During the first year, both the treatment and control groups had an increase in fasting glucose and insulin, and the increase was of similar magnitude in both groups (Guest *et al.* 1998). There was no mention of a relationship between the OGTT results and steroid dose.

Haffner has also reported on glucose tolerance in children with renal transplants, before and during rhGH treatment (Haffner *et al.* 1998b). He found that at baseline fasting glucose, insulin and HbA1c levels were higher in transplanted patients

compared to controls, and that fasting insulin levels were higher in the transplanted patients compared to CRI and dialysis patients. In this study fasting glucose was directly correlated with the dose of steroid, but fasting insulin was not. There was no change in glucose tolerance in transplanted patients receiving rhGH treatment, but insulin levels rose and remained elevated for up to 5 years (Haffner *et al.* 1998b).

It is difficult to explain the discrepancy between these three studies: baseline insulin values increased during rhGH treatment in Haffner's study; in our patients levels increased then returned to baseline; whilst in the study of Van Dop insulin was unchanged by treatment. Patient variation, in terms of steroid dose (total dose and frequency), other immunosuppressants, age, nutritional status, pubertal status, and other factors may play a part.

Perhaps a better measure is the effect of rhGH treatment on HbA1c in renal transplant patients. In our study, baseline HbA1c was higher in the transplanted group compared to the CRI group ($p = 0.003$) and to the combined dialysis groups ($p < 0.0001$). There was however no change in HbA1c during rhGH treatment in the transplanted group. In Haffner's study HbA1c was unchanged after 3 years of rhGH treatment (Haffner *et al.* 1998b). HbA1c was not measured in the other studies described above (Van Dop *et al.* 1991; Guest *et al.* 1998), but has been reported elsewhere to be unchanged by rhGH treatment after renal transplantation (Van Es *et al.* 1991; Jabs *et al.* 1993).

One child in the transplanted arm of our study developed glucose intolerance nine months after starting rhGH. Glucose tolerance returned to normal when rhGH was discontinued, however she later went on to develop diabetes mellitus requiring insulin treatment some months after stopping rhGH treatment. There have been other case reports of patients with renal transplants developing diabetes or glucose intolerance during rhGH treatment (Ingulli *et al.* 1993).

Conclusion

The data presented here show an increase in insulin secretion with rhGH treatment in CRF, but there was no effect on fasting glucose and no change in glycosylated haemoglobin levels in any of the groups. These results are similar to those reported for other groups of patients with CRF receiving rhGH, and indeed are similar to results during rhGH treatment of short normal patients and those with GHD. The duration of increased insulin secretion appears to be variable. Undoubtedly some CRF patients already have a degree of insulin insensitivity before treatment, and these patients may be at risk of overt glucose intolerance during rhGH treatment. Long-term follow up is necessary in CRF patients receiving rhGH treatment.

II. LIPIDS

Patients with CRF have an increased risk of developing cardiovascular disease in later life. This is thought to be due in part to abnormalities in lipid metabolism, which are known to be present in childhood (Querfeld 1993). Little is known of the natural history of uraemic dyslipidaemia in childhood, but lipid levels are often significantly elevated and treatment of hyperlipidaemia may be of therapeutic benefit. As discussed above, children with CRF also have insulin resistance, which is another risk factor for cardiovascular disease. As GH has actions on lipid metabolism as well as on carbohydrate metabolism, it is important to determine the effect of rhGH treatment on lipid profiles in children with CRF.

Growth hormone deficiency is characterised by reduced free fatty acid release by adipose tissue causing diminished ketogenesis. Growth hormone replacement restores lipolysis and ketone production. Administration of intravenous GH to normal subjects increases release of free fatty acids from adipose tissue (Rabinowitz *et al.* 1965), however two years of rhGH treatment in short normal children had no effect on fasting free fatty acids, triglyceride or cholesterol levels (Lesage *et al.* 1991). In this

study, fasting triglyceride and cholesterol levels before and during rhGH treatment were measured in different groups of CRF patients.

Methods

Fasting triglyceride and cholesterol were measured before treatment, after 1 week of rhGH treatment, then three monthly for one to two years in the patients attending the London centres, and three monthly in all other patients.

Results

Cholesterol and triglyceride levels during treatment in the different patient groups are shown in Table 10.3.

Cholesterol

At the start of treatment, mean cholesterol levels were at or just above the upper limit of the normal range in each group (Figure 10.13). The highest values were in the PD group, where the values were significantly higher than in the HD group ($p < 0.001$); 69% of PD patients had a cholesterol level above the normal range, compared with 11% of children in the HD group. Cholesterol was also higher in the transplanted group compared to the HD group ($p = 0.02$). Twenty-seven percent of transplanted children had a baseline cholesterol above normal, as did 21% of the CRI group and 33% of the infants. There were no significant differences between the groups.

Cholesterol levels were unchanged during treatment in the CRI, infant and dialysis groups, but there was a significant fall in cholesterol during the first year of rhGH treatment in the transplanted group as a whole (Figures 10.14 and 10.15). When the transplanted group was analysed by comparing the treatment and control groups, cholesterol levels fell in both groups, but not significantly so (control group $p = 0.27$; rhGH group $p = 0.45$, ANOVA), Figure 10.16. There was no difference between the fall in cholesterol in these groups, but interestingly, the decrease in cholesterol in the

control group during the year of rhGH (2nd year) was greater when compared with the first year of no treatment ($p = 0.002$), suggesting that rhGH had some effect on cholesterol levels.

Triglyceride

Mean fasting triglyceride was at or above the upper limit of the normal range in each group at the start of treatment (Figure 10.17). Seventy-eight percent of infants, 21% of CRI, 56% of PD, 86% of HD and 14% of transplanted patients had fasting triglycerides above the normal range. The highest values were found in the PD group; these were significantly higher than the CRI ($p = 0.034$) and the transplanted ($p = 0.0016$) groups. There was no difference between the PD and HD groups ($p = 0.42$), but mean triglyceride was lower in the transplanted group than in the HD group ($p = 0.002$). During treatment mean triglyceride rose in the CRI group ($p = 0.004$), but was unchanged in all the other groups (Figure 10.18 - 10.20).

Discussion

Abnormalities in lipid profile were evident in the majority of patients before treatment. The findings are similar to those found in adult patients on renal replacement therapy (Oda and Keane 1998). Cardiovascular and cerebrovascular disease are major causes of mortality in HD patients (US Renal Data System 1995), and death from cardiovascular disease is a major cause of graft loss in renal transplant patients (Lindholm *et al.* 1995). Whilst children on renal replacement therapy do not exhibit overt cardiovascular disease, it is likely that its origins start in childhood (Kari *et al.* 1997). The literature relating to lipid abnormalities in children on dialysis (Querfeld 1993) and after transplantation is limited (Sharma *et al.* 1994; Singh *et al.* 1998; Silverstein *et al.* 2000).

The main abnormality in CRF is decreased lipoprotein catabolism resulting in reduced clearance of intermediate particles and diminished formation of high density lipoproteins (Chan JK *et al.* 1981). This is due to reduced lipoprotein lipase and

hepatic triglyceride lipase activities (Chan *et al.* 1984). This is likely to be multifactorial in origin, but insulin resistance, hyperparathyroidism and uraemic inhibitors have been proposed as possible mechanisms.

The lipid abnormalities were most pronounced in the PD group, as is found in the adult CRF population (Oda and Keane 1998). Hypercholesterolaemia and hypertriglyceridaemia were both common in our patients; 69 % of the PD patients had a cholesterol value greater than the upper limit of normal, and 56% had a triglyceride value above normal. This compares with values of 74% and 50% quoted for the adult population (Oda and Keane 1998), and of 69% and 90% for paediatric PD patients in another study (Querfeld *et al.* 1988). This suggests that lipid abnormalities in CRF do start early in life, and raises the question of therapeutic intervention at this stage, even before signs of cardiovascular disease are evident (Fried *et al.* 1999). Even in the CRI group who have residual renal function, 21% had a raised cholesterol and 25% a raised triglyceride. Perhaps of more concern, 33% of the infants with CRI had a raised cholesterol, and 78% a raised triglyceride. These children were young with a mean age of only 1.9 years. Similar high results in young children have been reported previously (Querfeld *et al.* 1988).

Risk factors for hyperlipidaemia during PD include absorption of glucose from, and protein loss (including apoproteins), into the dialysate (Querfeld *et al.* 1991). In small children the surface area of the peritoneum relative to body size is greater than in older children and adults, and may contribute to the abnormalities found. Furthermore to sustain growth in children with CRF, high calorie feeds are administered. Often significant proportions of the calories in these feeds are derived from fat which is likely to be another contributing factor. The lipid profile of children treated with PD are of an atherogenic nature, with high concentrations of total cholesterol and triglycerides and an increase in very low and low density lipoproteins, with normal high density lipoproteins (Querfeld *et al.* 1991).

In the HD population, hypertriglyceridaemia was more of a problem than hypercholesterolaemia. Eighty-six percent of HD patients had raised triglycerides, and 11% raised cholesterol. Comparable figures for the adult population are 49% and 39% respectively (Oda and Keane 1998), and virtually identical results to ours have been reported previously in paediatric haemodialysis patients (Pennisi *et al.* 1976). The pathogenesis of the marked and consistent hypertriglyceridaemia that is reported in patients on haemodialysis is due to a combination of increased production of ApoB lipoprotein and reduced lipoprotein lipase activity (Kasiske and Keane 1991).

Treatment with rhGH had little effect on lipid levels. There was an increase in triglyceride in the CRI group during the year of rhGH treatment ($p = 0.004$, ANOVA). This was evident by 3 months and persisted through the year. In the absence of a control group it is difficult to know the significance of this finding, which may be due to an effect of time alone. Reports from other groups however report that rhGH has little effect on lipid profiles (Hokken-Koelega *et al.* 1991; Fine *et al.* 1994; Haffner *et al.* 1998b). The paper by Fine in 1994 reported a placebo controlled study of patients with CRI; there was no change in cholesterol or triglyceride in either group during the initial 2 years of the study (Fine *et al.* 1994). A later follow up report confirmed no change in cholesterol or triglyceride after 5 years of rhGH treatment (Fine *et al.* 1996b).

There are fewer data available for dialysed patients; rhGH treatment had no effect on lipid levels in the PD patients reported here. Similar data have become available since (Haffner *et al.* 1998b).

The transplanted patients exhibit a different pattern of dyslipidaemia, with hypercholesterolaemia being more of a problem. The potential risk factors in this population are immunosuppressive therapy, proteinuria and obesity, and there is increased lipoprotein production by the liver (Cattran *et al.* 1979). The proportion of our paediatric patients with hypercholesterolaemia was 32%, compared with 79% of adult renal transplant recipients (Oda and Keane 1998). One study of paediatric renal

transplant recipients at a mean time of 7.4 years post transplantation, found that 17 of 33 (52%) patients had hypercholesterolaemia (Sharma *et al.* 1994), another reported an incidence of hypercholesterolaemia of 51% (Pennisi *et al.* 1976). In both of these studies cholesterol levels correlated with the dose of prednisolone. In our study, we found no correlation with GFR ($r = -0.157$), prednisolone dose ($r = 0.255$), weight for height ($r = 0.326$, $p = 0.14$) nor with insulin ($r = -0.112$).

Yet another paediatric study reported that 52% of 62 transplanted patients had hypercholesterolemia (Silverstein *et al.* 2000). In this study the risk factors for having high cholesterol levels were pre-transplant hypercholesterolemia and years since transplantation. This study also reported triglyceride levels (Silverstein *et al.* 2000). Fifty-two percent of pediatric renal transplant recipients had elevated triglycerides, with the only demonstrable risk factor being reduced GFR (Silverstein *et al.* 2000). Oda reported similar data for adult renal transplant recipients, with 46% of the patients studied having high triglyceride levels (Oda and Keane 1998). Only 18% of our patients had a high plasma triglyceride level. It is difficult to explain these differences; one possibility could be the more frequent use of alternate day steroids in our patients, age, nutritional status, renal function or perhaps racial differences between the populations studied.

There was little change in lipid profiles in the transplanted group during rhGH treatment. There was a reduction in cholesterol, but when this was analysed further there was a fall in the control group as well as the treatment group, implying at least some effect due to time alone. Other groups have reported the effects of rhGH on lipid profiles in patients with renal transplants. All reported no change in either cholesterol or triglyceride levels (Fine *et al.* 1991b; Bartosh *et al.* 1992; Haffner *et al.* 1998b). Two years of rhGH treatment in short normal children had no effect on either cholesterol or triglyceride (Lesage *et al.* 1991).

Conclusion

Prior to treatment, significant numbers of children in each of the subgroups of the study had elevated cholesterol and triglyceride levels. During treatment with rhGH there was a rise in triglyceride levels in the CRI group but no other definitive changes were seen.

III. RENAL BONE DISEASE

Endogenous GH stimulates longitudinal bone growth both directly and through the actions of its mediator IGF-I as outlined in Chapter 1. Growth hormone treatment results in an increase in the number and activity of osteoblasts (Ernst and Froesch 1988), increasing bone length and bone mineral deposition. Growth hormone deficiency is associated with reduced longitudinal bone growth, reduced final adult height and reduced bone mineral content (Lu *et al.* 1992; Saggese *et al.* 1993). A significant proportion of accumulated bone mass occurs during puberty, such that normal duration and timing of puberty are required for this to be accomplished successfully.

As renal function declines there is reduced activation of vitamin D within the kidney, causing a fall in plasma calcium. If this is not corrected, the patient develops osteopenia. Hypocalcaemia (and hyperphosphataemia due to CRF) stimulate secretion of PTH; if left unchecked, hyperparathyroidism develops. Hyperparathyroidism will, with time, lead to osteitis fibrosa cystica (Hruska 2000). In the growing skeleton these consequences of CRF eventually lead to renal rickets. Furthermore, GH is involved in bone remodelling and children with CRF exhibit a degree of GH resistance (Chapter 8). Children with CRF also have a delayed and shortened puberty, when bone mass accrual is significant. Yet another risk factor for some of these children is the use of steroids. The effects of rhGH treatment on bone in CRF have not been fully evaluated.

Methods

Calcium, phosphate, and ALP were measured before, after one week of rhGH, then 3 monthly in the London patients. In all other patients, calcium, and ALP were measured 3 monthly; phosphate was recorded 3 monthly in the infants, CRI and transplanted patients. Parathyroid hormone was measured 3 monthly in all patients. Analysis of change in parameters during the study was by ANOVA.

Results

Data for calcium, phosphate, ALP and PTH are given in Tables 10.4 and 10.5.

Calcium

There was no difference between plasma calcium in the different subgroups at the start of treatment (Figure 10.21). There was no significant change in calcium during the study in any of the patient groups (Figure 10.22 - 10.24).

Phosphate

Phosphate results are available for the infants, CRI and transplanted patients. Phosphate was significantly lower in the transplanted group than the CRI group ($p = 0.04$), Figure 10.25. There were significant increases in phosphate levels during rhGH treatment in the CRI and transplanted patients that were both highly significant (Figure 10.26). Comparing the transplant control and treatment groups, an increase was seen in the treatment group ($p = 0.02$) but not the control group ($p = 0.41$), Figure 10.27.

Alkaline Phosphatase

Alkaline phosphatase data are shown in Figures 10.28 - 10.31. At the start of the study, ALP was highest in the dialysis groups and lowest in the transplanted group. There was a highly significant increase in ALP in the transplanted group during rhGH treatment ($p < 0.0001$), Figure 10.29. Comparing the transplant control and treatment groups, an increase was seen in the treatment group ($p < 0.0001$) but not the control

group ($p = 0.55$), Figure 10.31. A significant increase in ALP was also seen in the PD group, but not in any of the other groups.

Parathyroid Hormone

Results for PTH are given in Table 10.5 and Figures 10.32 - 10.35. Before treatment, PTH was highest in the HD group, being significantly higher than the CRI ($p < 0.001$), the infant ($p = 0.03$), the transplanted ($p < 0.001$), and the PD ($p = 0.001$) groups, Figure 10.32. The mean PTH in the CRI group was above the normal range, whilst that in the transplanted group was within the normal range, but there was only a trend for higher levels in the CRI compared to the transplanted group ($p = 0.09$). Parathyroid hormone levels in the infant group were higher than in the CRI ($p = 0.005$), the transplanted ($p < 0.001$) and the PD groups ($p = 0.04$).

During rhGH treatment, PTH increased in the CRI group ($p = 0.01$), and decreased in the infant group ($p = 0.03$). Parathyroid hormone increased in the treatment group in the transplant arm of the study ($p = 0.05$), but not in the control group ($p = 0.35$). When PTH was analysed in all transplanted children during their first year of rhGH, there was however no significant change ($p = 0.13$).

Discussion

Abnormal renal function has a deleterious effect on bone metabolism giving rise to hyperparathyroidism and renal osteodystrophy. In the growing child renal osteodystrophy can result in renal rickets. Severe renal osteodystrophy may retard growth, and correction of renal osteodystrophy has been reported to improve growth by some (Chan JCM *et al.* 1981) but not other authors (Chesney 1985). The question arises as to whether rhGH treatment is effective in the presence of renal osteodystrophy, or whether rhGH treatment might worsen pre-existing renal osteodystrophy.

Chronic Renal Insufficiency

There was a significant increase in serum phosphate in the CRI patients. Growth hormone acts on the renal tubule to increase the tubular reabsorption of phosphate (Corvilian *et al.* 1962). However in renal failure there is already an accumulation of phosphate due to reduced tubular phosphate secretion so a further increase in phosphate might not have been expected. There was a small increase in PTH which could be secondary to the increase in phosphate. Nearly all of these children were on activated vitamin supplements at the start of the study.

There was no significant change in ALP in the CRI group, which is contrary to other published studies. Fine reported a two year placebo controlled trial of rhGH in CRI. In his study, there was no change in either calcium or phosphate, but after 1 year ALP was increased in the treatment group compared to the control group, but by 2 years there was no significant difference (Fine *et al.* 1994). Parathyroid hormone levels were not reported. Increase in ALP has been shown to be a good indicator of the growth response to treatment in other groups of children treated with rhGH, particularly if the bone ALP isoform is measured (Crofton *et al.* 1995).

There are several assays used for the measurement of ALP, so it is difficult to compare different studies. The ALP results in our CRI group are high compared to normal children and may explain why there was no further increase. There was however a trend for an increase, and the results are skewed by one patient who had a very high ALP at the start of the study.

Dialysis

The growth response in the dialysis groups was less than that seen in the CRI or transplanted groups. Despite a high ALP at baseline, a further increase was seen during rhGH treatment in the PD group. There was no significant change in PTH in either the PD or HD group. These children have more disturbed bone metabolism, which may be one reason for the poorer growth response to GH. One of the children

in the prepubertal PD group was withdrawn from the study after 11 months of rhGH treatment because of worsening renal bone disease. His calcium, phosphate, ALP and PTH were all within normal limits at the start of the study but increased steadily during rhGH treatment. It is possible that rhGH treatment was responsible, although this cannot be proven. Unfortunately no follow up data are available.

Transplantation

In the transplanted patients, there were small but significant increases in phosphate, PTH and ALP during rhGH treatment. The majority of patients with renal transplants have better renal function than patients with CRI, and phosphate, PTH and ALP were lower at baseline in this group compared to the other groups. They would therefore be expected to have less marked renal osteodystrophy, but virtually all of these patients receive treatment with steroids, which may have a detrimental effect. Patients with normal renal function who receive rhGH treatment show a marked increase in ALP which is said to correlate with the magnitude of the growth response (Crofton *et al.* 1995). There are few data reporting the effect of rhGH on PTH in normal children.

The increase in phosphate and ALP are not unexpected. The increase in PTH is slightly concerning, however when all 22 of the transplanted children were analysed during their first year of rhGH treatment, there was no longer any significant change in PTH. Hokken-Koelega reported similar data; in a placebo controlled trial of rhGH in prepubertal renal transplant recipients, ALP increased in the group receiving treatment but not in the control group. Parathyroid hormone was unchanged in both groups (Hokken-Koelega *et al.* 1996). Tonshoff reported a rise in ALP during the first year of rhGH treatment in 13 patients with renal transplants, but ALP was no different from baseline in the 10 patients who were followed for 3 years (Tonshoff *et al.* 1993).

Nine children in this study received rhGH for 2 years. There was no difference in phosphate [day 1:1.35(0.42)mmol/l; 2 yrs: 1.4(0.31), $p = 0.33$], ALP [day1:

227(75)U/l; 2 yrs: 273(97), $p = 0.12$] and PTH [day1: 4.8(3.4)pmol/l; 2 yrs:5.6(3.7), $p = 0.55$] after 2 years of rhGH treatment in these 9 children.

Renal Bone Disease

Other abnormalities of bone are also reported in CRF and following renal transplantation, namely slipped capital femoral epiphysis (Mehls *et al.* 1975) and avascular necrosis of the femoral head (Catterall and Roberts 1971; Mehls *et al.* 1981). There is also an increased risk of both of these conditions in patients with GHD. The effects of rhGH treatment on the course of these two conditions is unclear at present. There has been no systematic study of slipped capital femoral epiphysis or avascular necrosis in renal patients either before or during rhGH treatment. These conditions have been diagnosed during rhGH treatment of patients with CRF (Watkins 1996), but in many patients there were no x-ray films available from before treatment for comparison. The present study did not set out to address this particular issue.

Bone Mineral Content

There are two recent published studies reporting the effect of uraemia on bone mineral content. One study reported 13 prepubertal patients with CRI who were found to have reduced bone mineral content and bone mineral density compared to controls, and who had an increase in both of these parameters following 1 year of rhGH treatment (Lanes *et al.* 1996). Another study found bone mineral density to be no different to controls in 36 prepubertal children with CRF. Growth hormone treatment had no effect on bone mineral density in this group of patients (Boot *et al.* 1996). A recent abstract has compared children with CRI with both height- and age-matched controls, before and after 1 year of rhGH treatment (Gyssels *et al.* 2000). Before treatment total bone mineral content, but not total bone mineral density, was lower than age-matched controls, whereas there was no difference compared to

height-matched controls. After 1 year of rhGH treatment, there was an increase in both bone mineral content and bone mineral density (Gyssels *et al.* 2000).

Conclusion

There was, as expected, evidence of abnormal bone metabolism in all groups of patients at the start of the study. There were transient increases in phosphate and ALP in nearly all groups and an increase in PTH in the CRI group. There were no major changes in bone metabolism as a result of rhGH treatment, but continued follow up is required. One child in the dialysis group developed biochemical evidence of marked renal osteodystrophy during rhGH treatment.

Table 10.1a Fasting glucose (mmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	4.8 (3.9 - 5.6)	-	4.7 (3.8 - 5.5)	4.6 (3.5 - 5.8)	4.9 (4.0 - 7.0)	4.9 (4.0 - 6.3)	<i>p</i> = 0.84
CRI	4.7 (2.4 - 7.1)	4.9 (3.8 - 5.9)	4.9 (2.7 - 5.8)	4.9 (3.2 - 6.0)	4.8 (4.1 - 5.5)	5.0 (2.7 - 6.4)	<i>p</i> = 0.21
PD	4.9 (3.9 - 6.6)	-	5.3 (3.5 - 7.4)	5.5 (4.8 - 6.8)	4.8 (4.2 - 5.3)	4.9 (4.5 - 5.3)	<i>p</i> = 0.38
HD	5.3 (4.5 - 7.4)	-	6.4 (4.9 - 11.9)	5.0 (3.6 - 6.7)	5.8 (4.6 - 7.8)	4.8 (3.5 - 5.7)	<i>p</i> = 0.29
Transplant	4.8 (3.4 - 7.4)	5.4 (4.1 - 6.6)	5.4 (4.3 - 8.2)	4.9 (3.5 - 6.8)	5.0 (3.9 - 6.4)	4.8 (3.6 - 6.4)	<i>p</i> = 0.13

Normal range 2.5 - 5.3 mmol/l

Table 10.1b Fasting insulin (mU/l) during rhGH treatment in the infant, CRI, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	21.6 (4.3 - 68.0)		28.2 (7.0 - 93.0)	41.6 (4.0 - 75.0)	37.2 (9.0 - 69.5)	49.1 (6.1 - 92)	
CRI	9.3 (3.5 - 26.9)	13.5 (6.3 - 37.1)	12.7 (5.5 - 26.6)	10.6 (4.7 - 18.3)	11.3 (4.8 - 19.8)	10.6 (5.0 - 15.2)	<i>p</i> = 0.11
Transplant	15.7 (3.4 - 46.6)	34.0 (6.5 - 96.8)	52.4 (7.8 - 358.5)	21.0 (5 - 51.6)	30.0 (4.2 - 117.7)	22.5 (3.1 - 73.6)	<i>p</i> = 0.13

Normal range 3 - 17 mU/l

Table 10.2 HbA1c (%) during rhGH treatment in the CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
CRI	3.6 (1.7 - 7.0)	-	3.5 (2.0 - 6.4)	3.5 (1.9 - 6.2)	3.7 (2.1 - 6.2)	3.7 (2.0 - 6.4)	$p = 0.14$
PD	3.9 (2.2 - 8.1)	-	3.2 (2.6 - 3.7)	2.8 (2.1 - 3.9)	3.0 (2.7 - 3.2)	4.7 (2.4 - 8.5)	$p = 0.29$
HD	3.0 (1.8 - 5.4)	-	2.6 (2.4 - 2.8)	2.5 (1.9 - 3.3)	2. (1.9 - 2.4)	2.4 (1.6 - 3.2)	$p = 0.49$
Transplant	4.6 (3.3 - 6.0)	-	4.6 (3.1 - 6.2)	4.6 (3.2 - 6.3)	4.8 (2.8 - 7.9)	5.0 (2.9 - 6.7)	$p = 0.27$

Normal range 2.5 - 6.0 %

Table 10.3a Fasting cholesterol (mmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	5.7 (3.3 - 8.7)	-	4.8 (3.3 - 6.2)	5.4 (3.8 - 7.3)	4.8 (3.2 - 7.3)	4.8 (2.9 - 8.2)	
CRI	5.8 (3.0 - 16.1)	6.2 (3.8 - 14.9)	6.0 (3.3 - 18.4)	6.4 (3.2 - 27.6)	6.5 (3.1 - 21.7)	5.7 (3.7 - 18.1)	<i>p</i> = 0.18
PD	6.8 (4.0 - 9.6)	-	6.6 (4.0 - 8.5)	6.5 (4.2 - 9.9)	6.4 (4.0 - 7.9)	6.3 (5.2 - 7.4)	<i>p</i> = 0.14
HD	4.7 (3.7 - 6.4)	-	5.0 (4.0 - 6.1)	4.9 (3.6 - 6.8)	4.9 (3.7 - 6.6)	4.5 (3.2 - 6.5)	<i>p</i> = 0.69
Transplant	5.6 (3.9 - 8.1)	5.7 (3.4 - 7.5)	5.3 (3.8 - 7.5)	5.5 (3.2 - 8.2)	5.1 (3.5 - 8.1)	5.3 (3.8 - 8.2)	<i>p</i> = 0.008

Normal range 3.0 - 6.1 mmol/l

Table 10.3b Fasting triglyceride (mmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	4.7 (1.7 - 10.3)	-	2.8 (1.4 - 5.1)	3.0 (1.2 - 6.5)	1.3 (0.7 - 1.9)	2.0 (1.1 - 3.2)	
CRI	2.0 (0.6 - 7.7)	2.6 (0.7 - 7.2)	2.7 (0.8 - 10.9)	2.3 (0.5 - 10.7)	2.1 (0.5 - 11.3)	2.4 (0.7 - 11.3)	<i>p</i> = 0.004
PD	3.3 (1.1 - 9.6)	-	4.3 (1.9 - 9.5)	4.1 (1.6 - 9.9)	3.4 (1.2 - 7.9)	3.9 (1.8 - 10.6)	<i>p</i> = 0.37
HD	2.6 (1.4 - 3.6)	-	3.2 (1.7 - 4.6)	3.2 (0.9 - 5.6)	3.0 (1.0 - 6.2)	2.8 (0.9 - 5.5)	<i>p</i> = 0.93
Transplant	1.6 (0.5 - 3.2)	1.9 (0.9 - 3.7)	1.5 (0.5 - 3.1)	1.7 (0.7 - 4.3)	1.7 (0.7 - 4.4)	1.5 (0.6 - 5.0)	<i>p</i> = 0.58

Normal range 0.7 - 2.2 mmol/l

Table 10.4a Plasma calcium (mmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	2.53 (2.31 - 2.74)	-	2.53 (2.21 - 2.70)	2.56 (2.52 - 2.79)	2.64 (2.47 - 2.98)	2.52 (2.44 - 2.59)	$p = 0.31$
CRI	2.48 (2.00 - 2.80)	2.50 (2.13 - 2.98)	2.52 (2.14 - 3.33)	2.47 (2.20 - 2.74)	2.47 (2.08 - 2.68)	2.44 (2.15 - 2.68)	$p = 0.25$
PD	2.53 (2.21 - 2.92)	-	2.45 (2.18 - 2.80)	2.48 (2.07 - 2.78)	2.48 (2.17 - 3.06)	2.53 (2.14 - 2.90)	$p = 0.66$
HD	2.42 (2.23 - 2.53)	-	2.34 (1.75 - 2.51)	2.31 (1.69 - 2.51)	2.55 (2.12 - 2.96)	2.36 (1.79 - 2.60)	$p = 0.14$
Transplant	2.42 (2.10 - 2.55)	2.46 (2.17 - 2.67)	2.44 (2.13 - 2.68)	2.42 (2.18 - 2.67)	2.45 (2.21 - 2.61)	2.48 (2.27 - 2.84)	$p = 0.23$

Normal range 2.26 - 2.62 mmol/l

Table 10.4b Plasma phosphate (mmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	1.48 (1.10 - 1.96)	-	1.50 (1.10 - 2.10)	1.60 (1.40 - 2.10)	1.65 (1.36 - 2.00)	1.38 (1.22 - 1.50)	$p = 0.73$
CRI	1.53 (1.03 - 2.17)	1.58 (1.16 - 2.25)	1.69 (1.23 - 2.140)	1.64 (1.20 - 2.14)	1.70 (1.14 - 2.30)	1.73 (1.13 - 2.30)	$p < 0.001$
Transplant	1.34 (0.92 - 2.13)	1.39 (0.86 - 1.89)	1.57 (1.06 - 2.37)	1.52 (1.21 - 1.92)	1.56 (1.13 - 1.98)	1.62 (1.25 - 1.98)	$p < 0.001$

Normal range 0.90 - 1.80 mmol/l

Table 10.5a Alkaline phosphatase (U/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	445 (178 - 870)	-	554 (199 - 1409)	474 (265 - 645)	403 (329 - 538)	576 (270 - 1118)	$p = 0.43$
CRI	299 (125 - 2207)	234 (116 - 797)	321 (141 - 593)	329 (123 - 622)	336 (109 - 790)	305 (140 - 647)	$p = 0.98$
PD	433 (92 - 874)	-	633 (235 - 1688)	619 (223 - 1188)	598 (204 - 1386)	764 (212 - 1662)	$p = 0.028$
HD	552 (248 - 1501)	-	708 (286 - 1252)	662 (277 - 894)	661 (255 - 1250)	766 (256 - 1590)	$p = 0.77$
Transplant	213 (95 - 523)	172 (77 - 426)	351 (126 - 1020)	349 (110 - 1094)	335 (149 - 722)	303 (99 - 552)	

Normal range 150 - 450 U/l

Table 10.5b Parathyroid hormone (pmol/l) during rhGH treatment in the infant, CRI, PD, HD, and transplanted groups

	Day 1	Day 8	3 Months	6 Months	9 Months	1 Year	ANOVA
Infants	14.5 (2.9 - 36.3)	-	13.4 (4.1 - 31.3)	13.3 (2.3 - 47.3)	4.1 (2.1 - 10.0)	7.2 (2.0 - 19.1)	$p = 0.026$
CRI	7.0 (0.5 - 20.4)	-	6.8 (1.5 - 19.9)	11.9 (1.3 - 71.5)	11.7 (1.5 - 30)	13.5 (1.5 - 53.5)	$p = 0.012$
PD	7.3 (0.8 - 21.3)	-	14.4 (1.0 - 49.4)	14.9 (2.1 - 76.5)	15.9 (1.3 - 73.4)	31.1 (1.2 - 125.0)	$p = 0.37$
HD	40.9 (3.1 - 103.0)	-	57.8 (4.0 - 136.8)	38.3 (8.1 - 78.9)	53.3 (2. - 122.8)	36.2 (1.8 - 112)	$p = 0.31$
Transplant	5.0 (1.7 - 13.2)	11.1 (1.3 - 98.8)	6.5 (1.5 - 26.7)	7.2 (1.8 - 23.4)	4.7 (0.1 - 10.9)	6.0 (1.4 - 20.7)	$p = 0.15$

Normal range 1 - 6.5 pmol/l

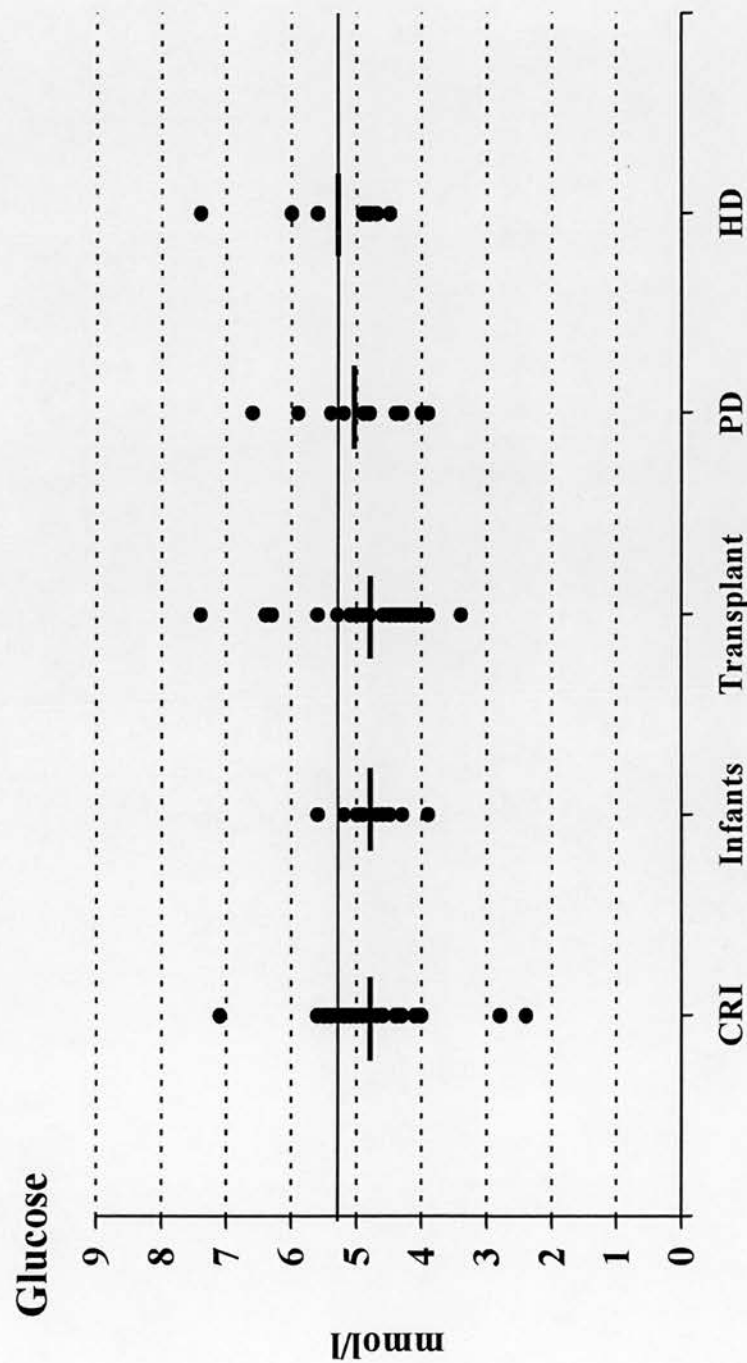


Figure 10.1 Fasting blood glucose at the start of the study in the CRI, infant, transplanted, peritoneal dialysis (PD) and haemodialysis (HD) groups. The upper limit of normal is indicated by the solid line.

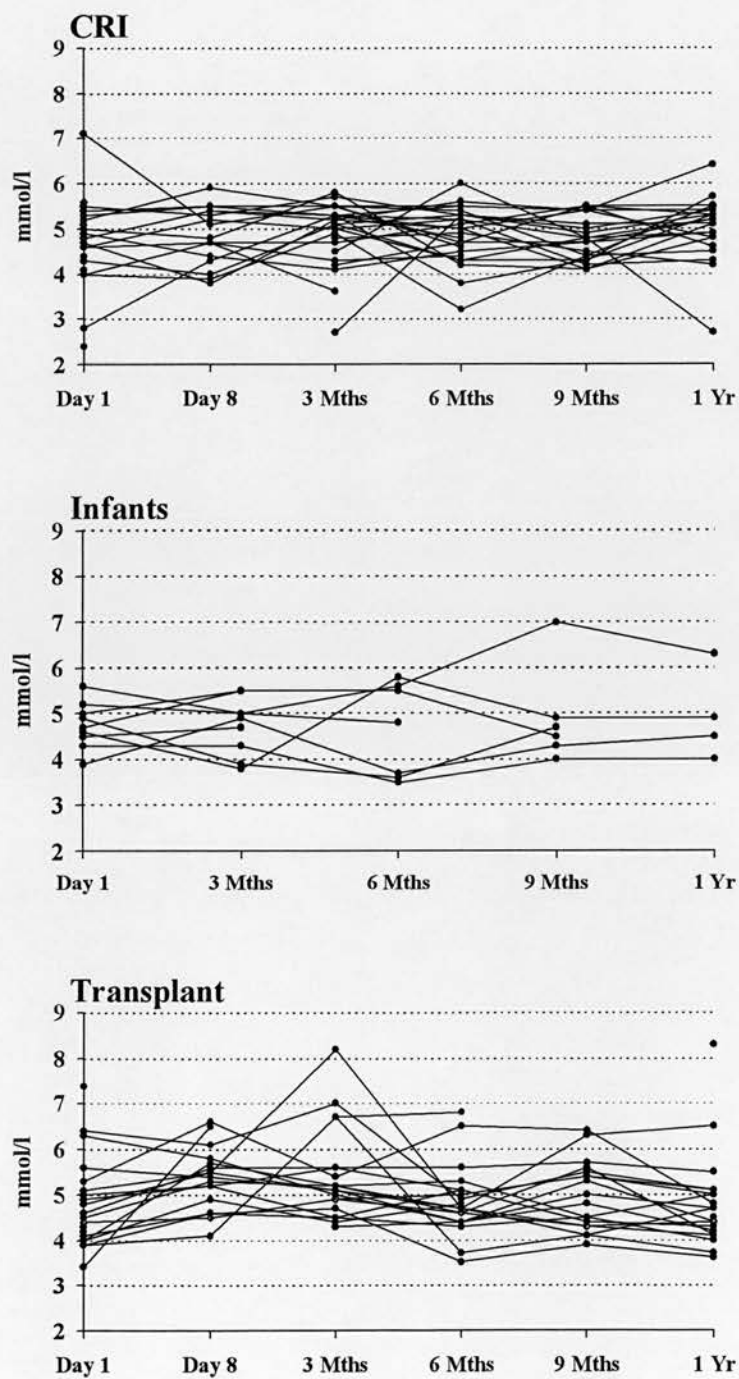


Figure 10.2 Fasting blood glucose during rhGH treatment in the CRI, infant and transplanted groups.

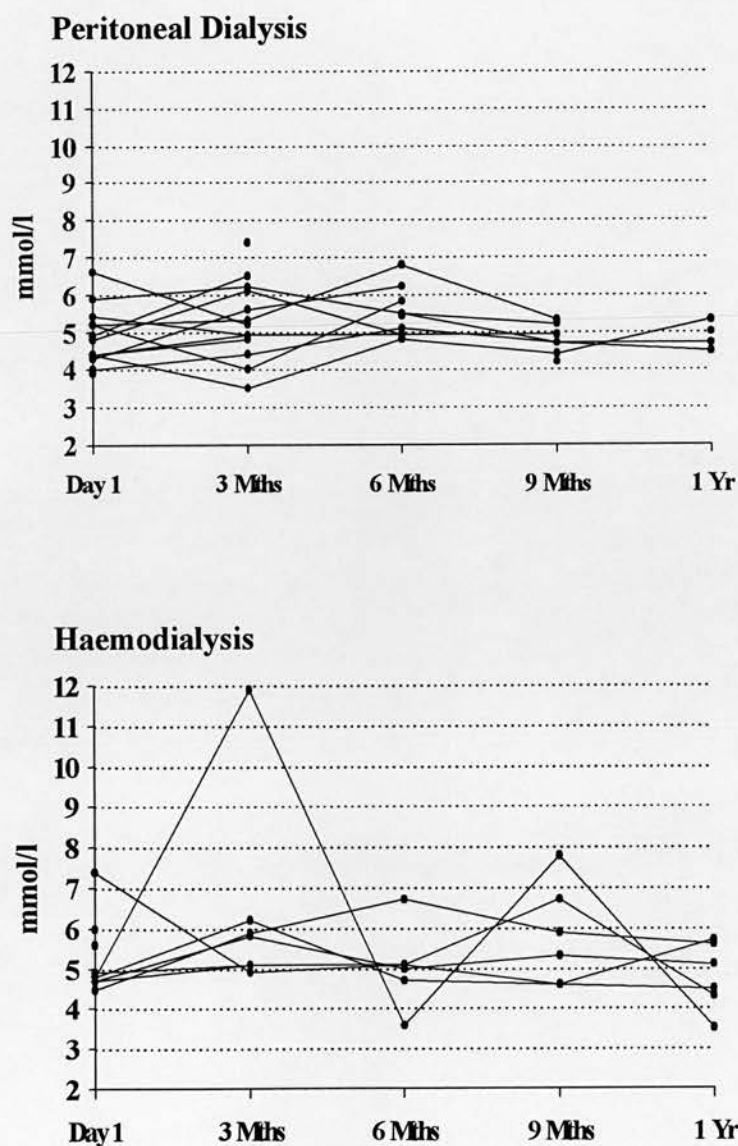


Figure 10.3 Fasting blood glucose during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

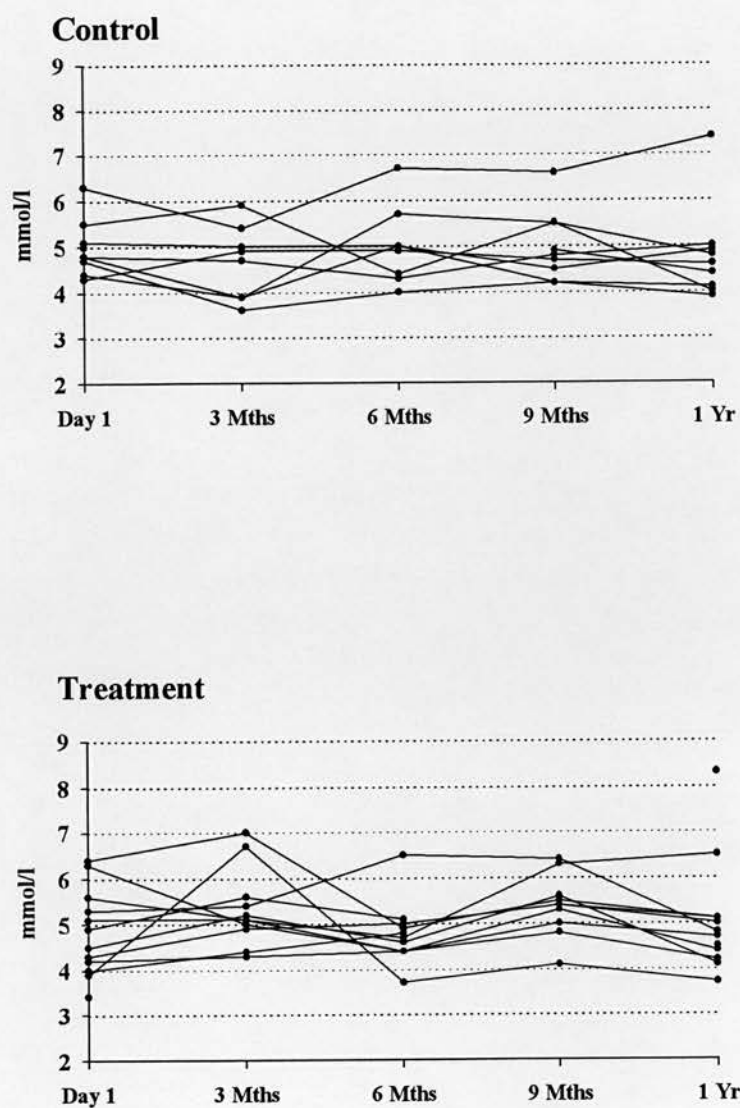


Figure 10.4 Fasting blood glucose during the first year of study in the transplanted control and treatment groups.

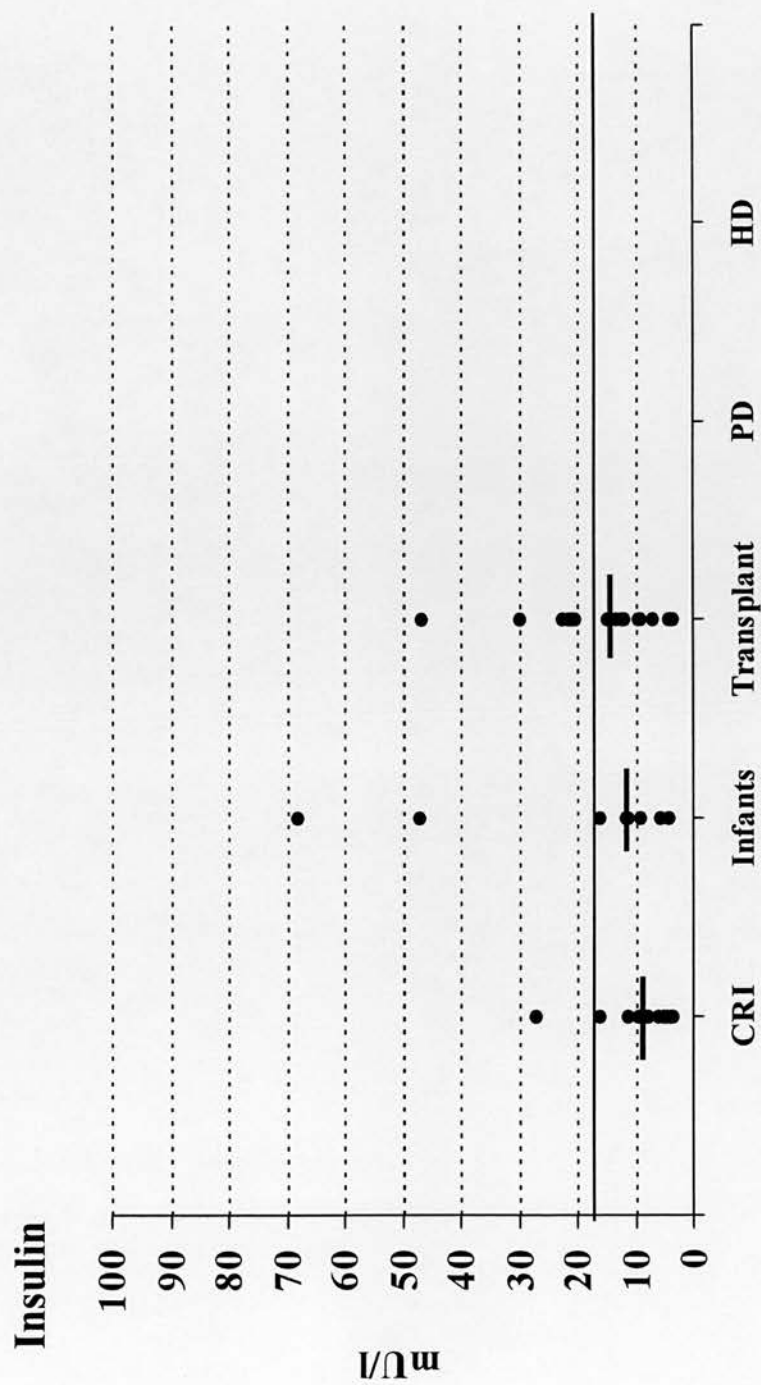


Figure 10.5 Fasting insulin at the start of the study in the CRI, infant and transplanted groups. The upper limit of normal is indicated by the solid line.

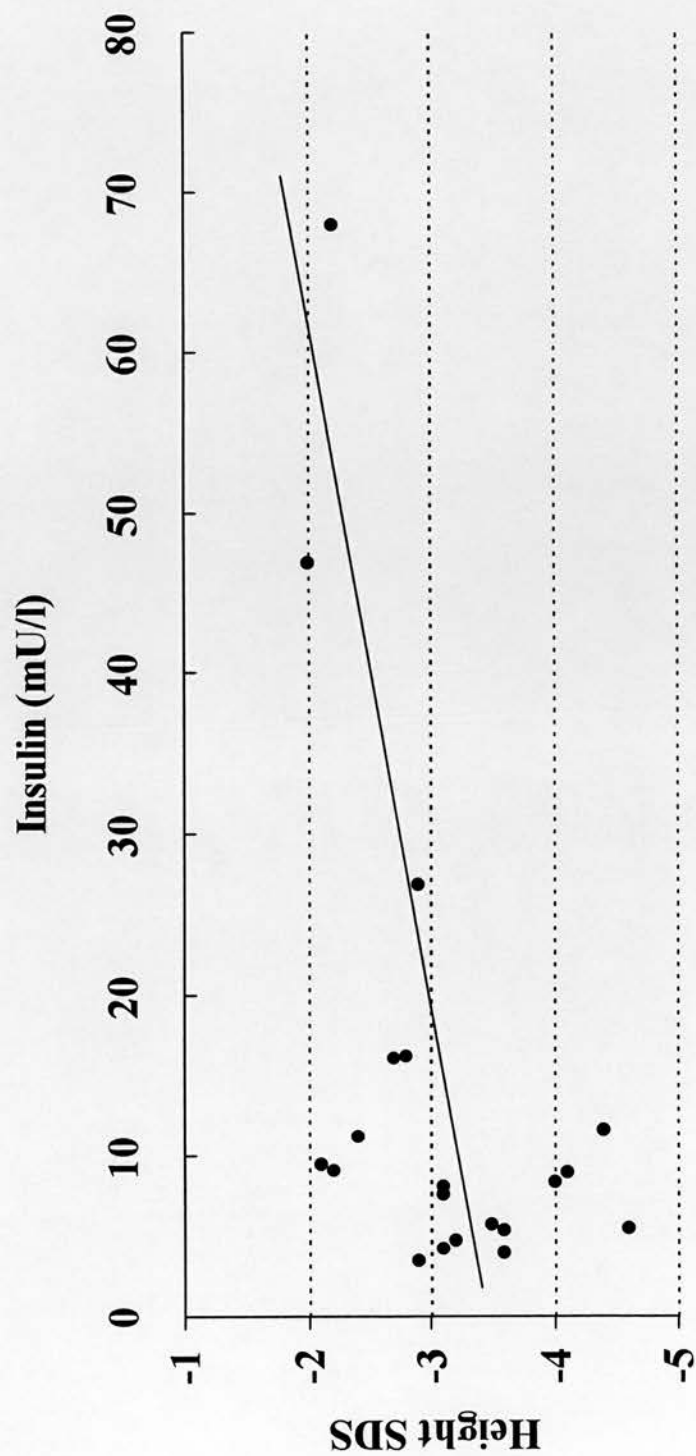


Figure 10.6 Fasting insulin at baseline plotted against height SDS on day 1 in the CRI group. $r = 0.491$, $p = 0.03$.

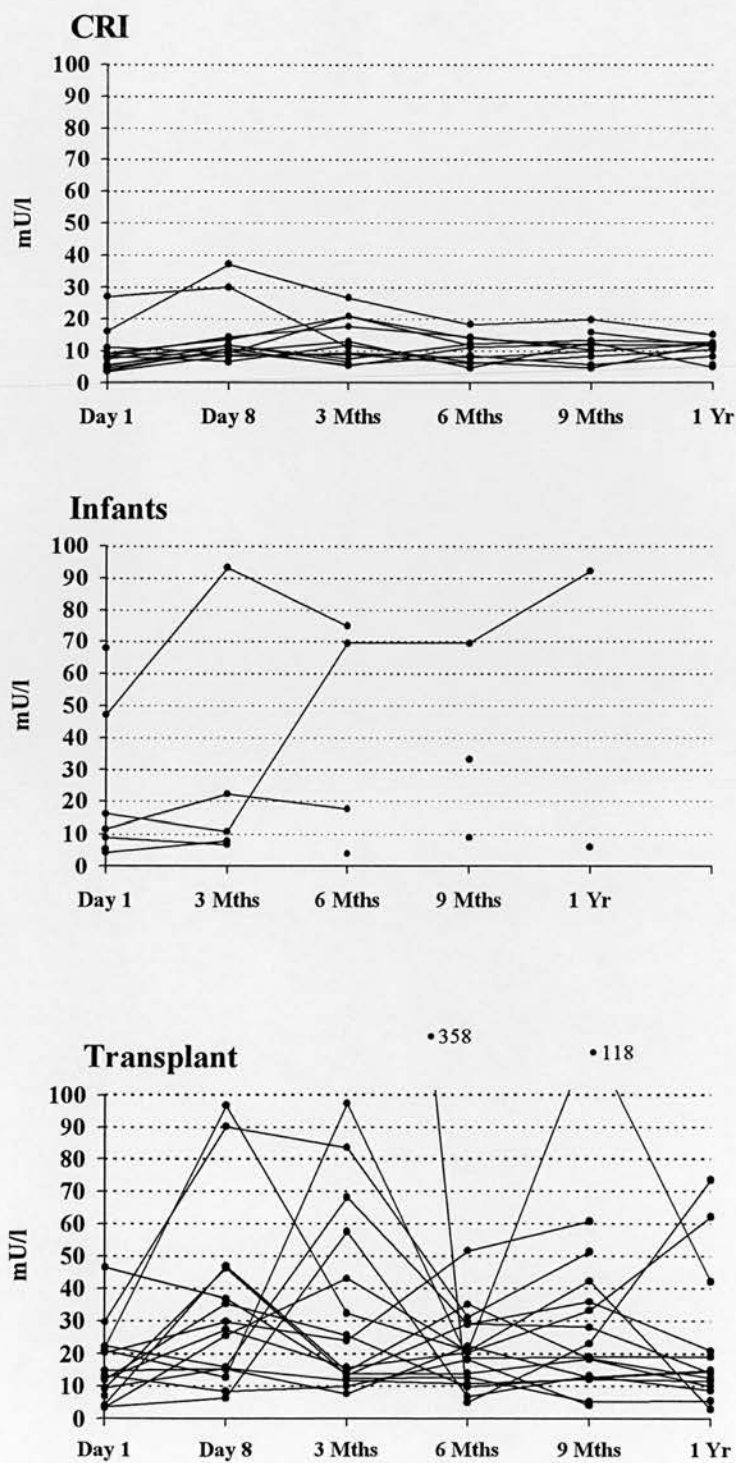


Figure 10.7 Fasting insulin during rhGH treatment in the CRI, infant and transplant groups.

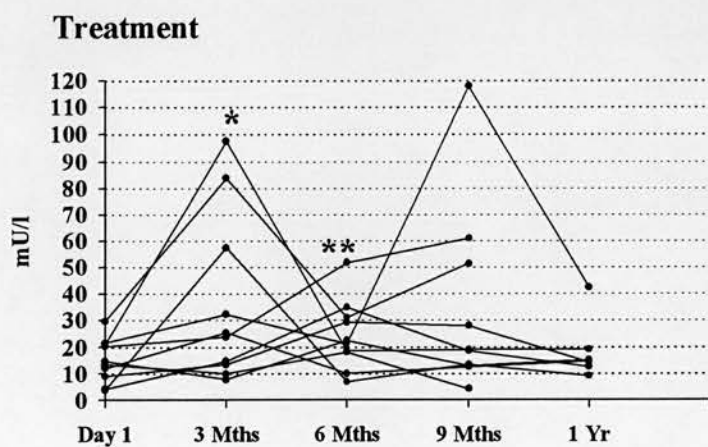
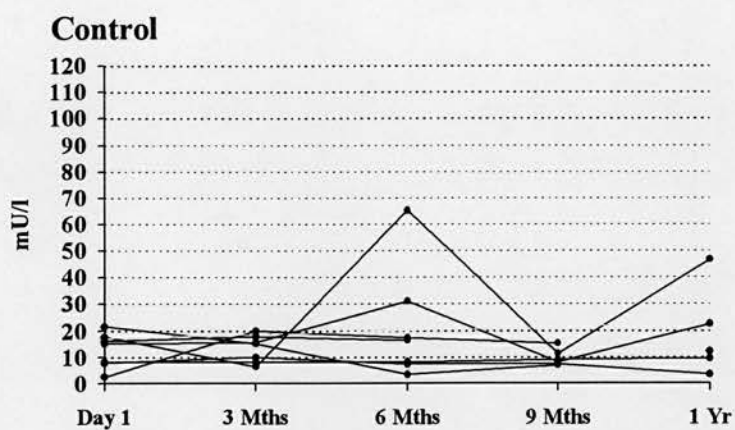


Figure 10.8 Fasting insulin during the first year of study in the transplant control and treatment groups. * $p = 0.04$ vs. day 1, ** $p = 0.05$ vs. day 1

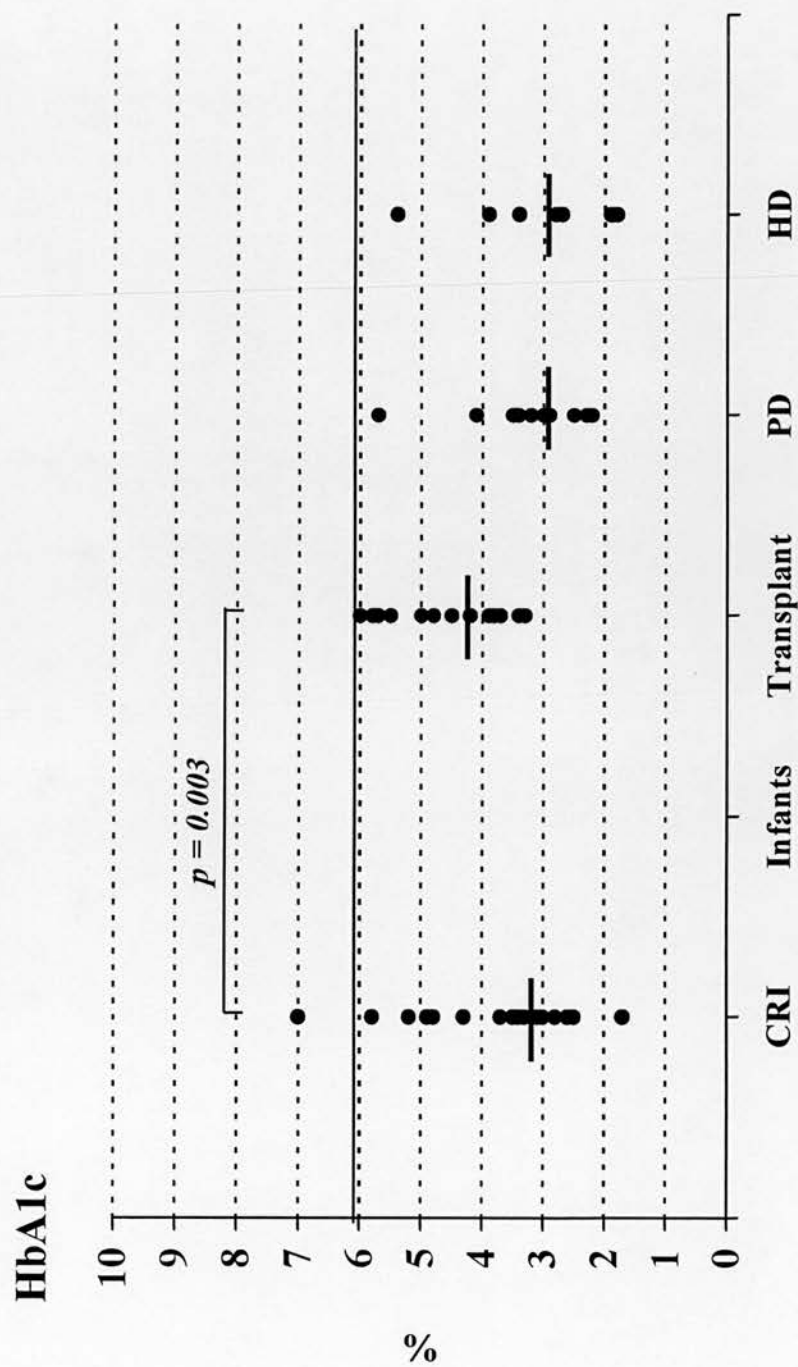


Figure 10.9 HbA1c at the start of the study in the CRI, transplant, peritoneal dialysis (PD) and haemodialysis (HD) groups. Differences between the groups are as shown. The upper limit of normal is indicated by the solid line.

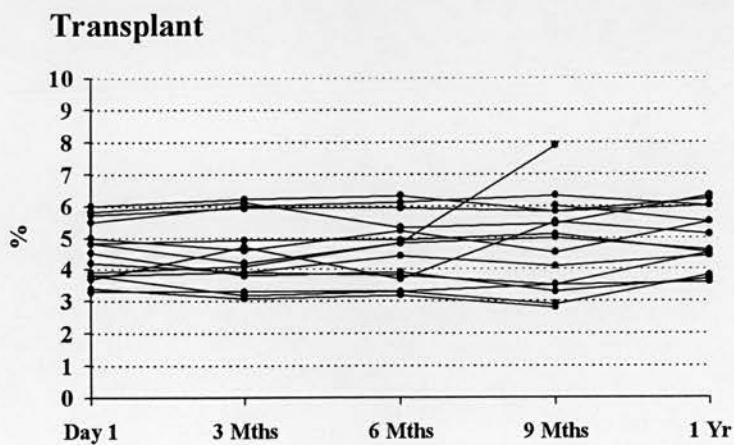
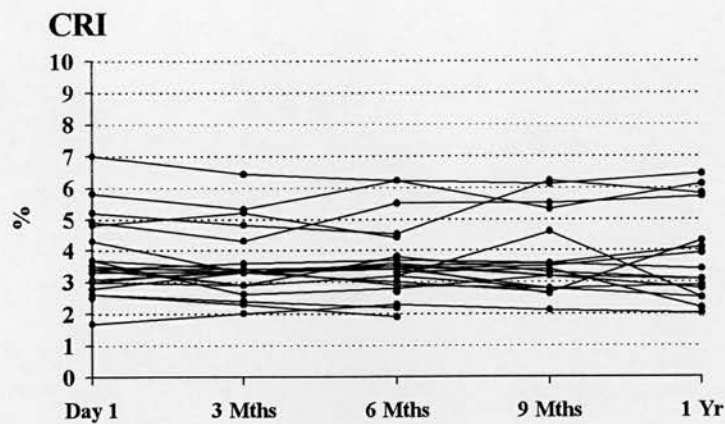


Figure 10.10 HbA1c during rhGH treatment in the CRI and transplant groups.

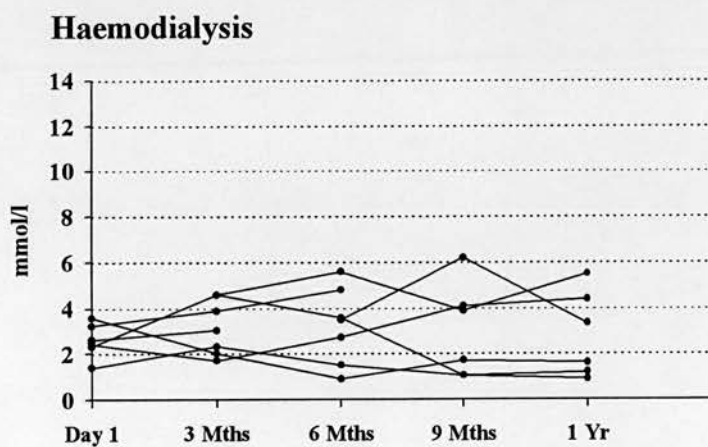
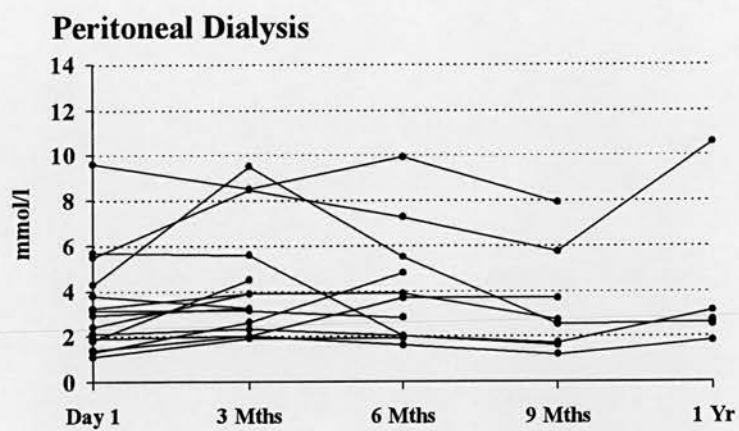


Figure 10.11 HbA1c during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

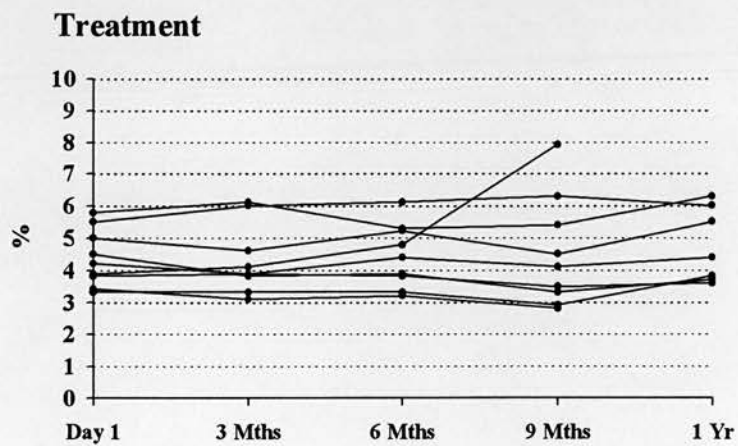
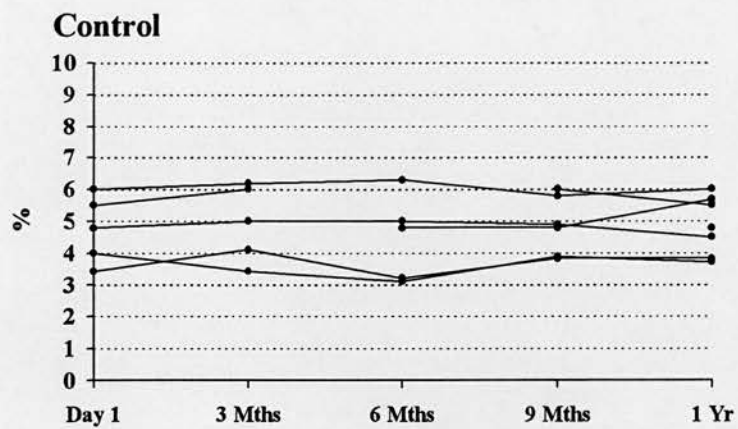


Figure 10.12 HbA1c during rhGH treatment in the transplant control and treatment groups.

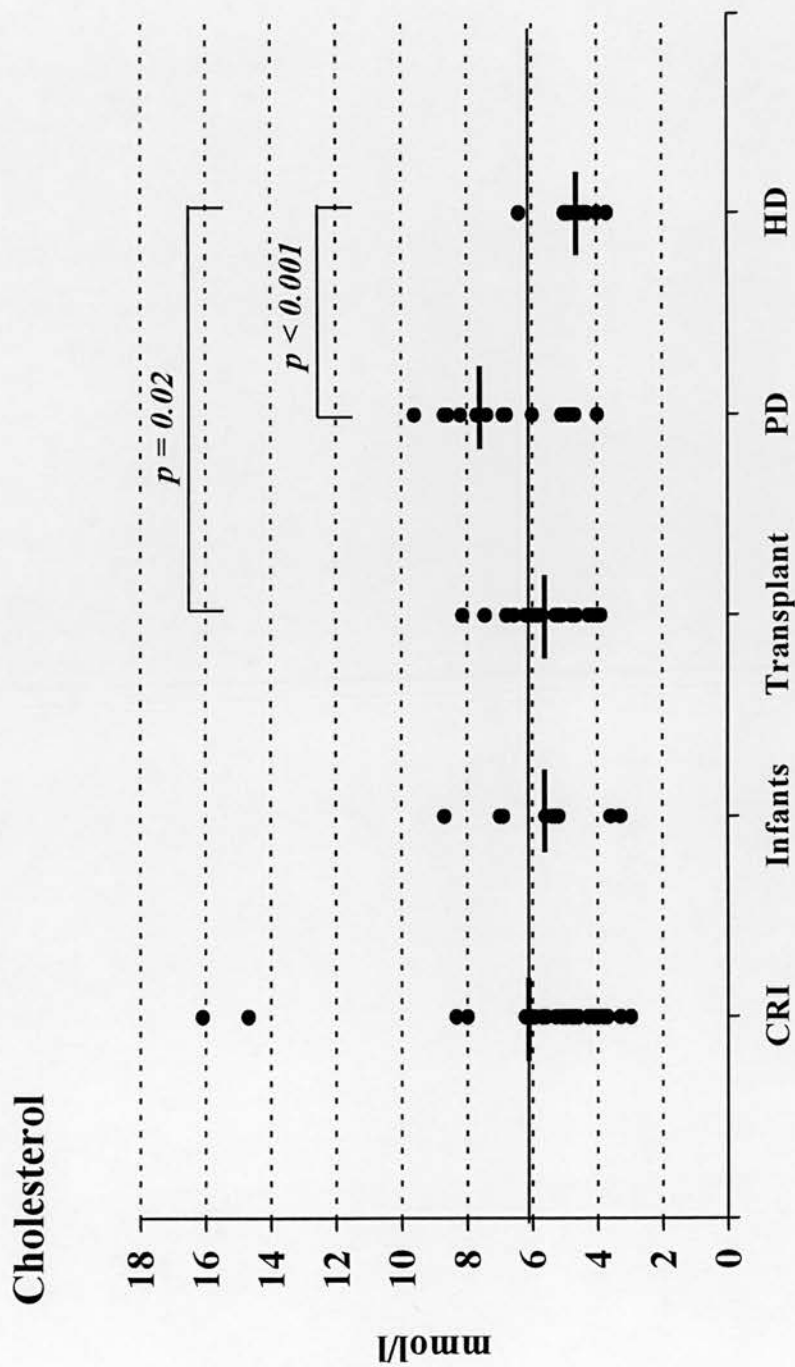


Figure 10.13 Fasting cholesterol at the start of the study in the CRI, infant, transplant, peritoneal dialysis (PD) and haemodialysis (HD) groups. Differences between the groups are as shown. The upper limit of normal is indicated by the solid line.

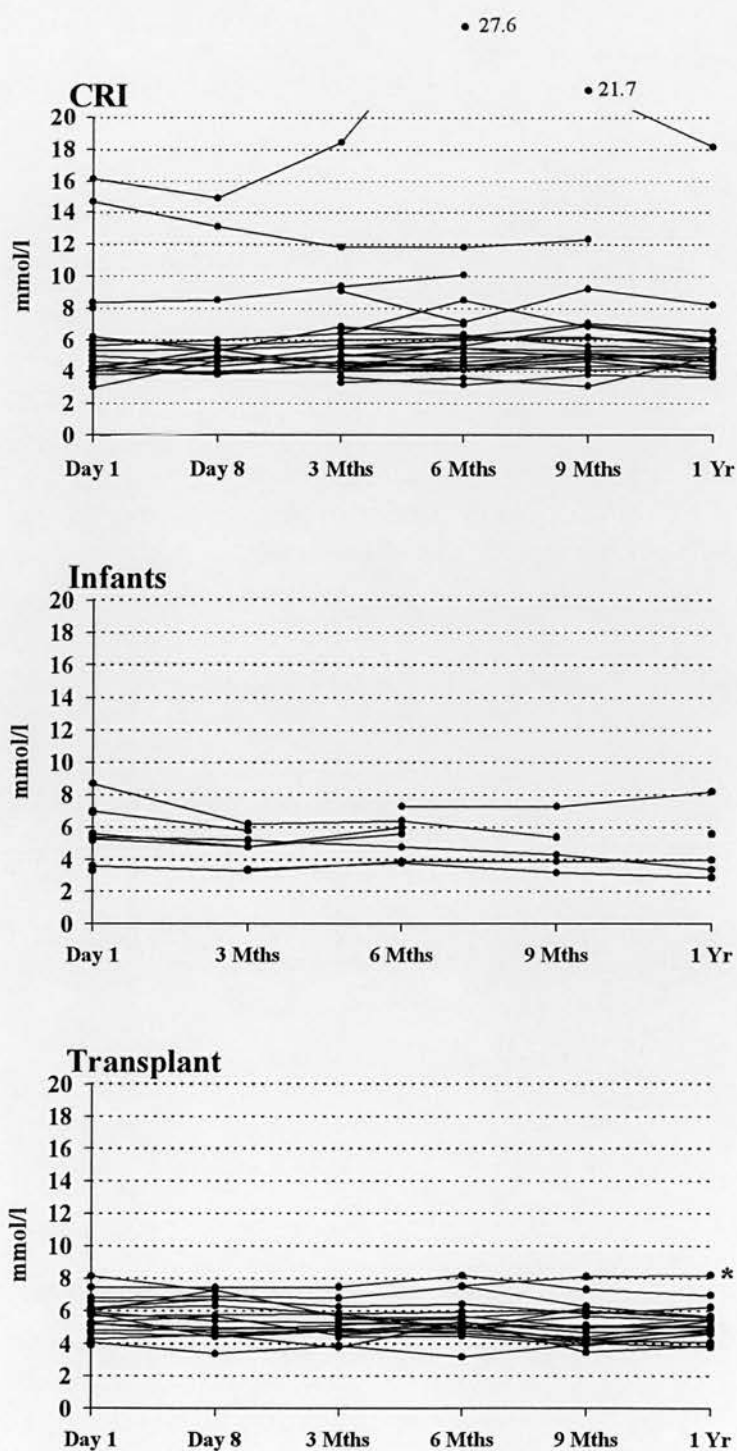


Figure 10.14 Fasting cholesterol during rhGH treatment in the CRI, infant and transplant groups. * Mean cholesterol decreased during rhGH treatment in the transplanted group, $p = 0.008$ ANOVA.

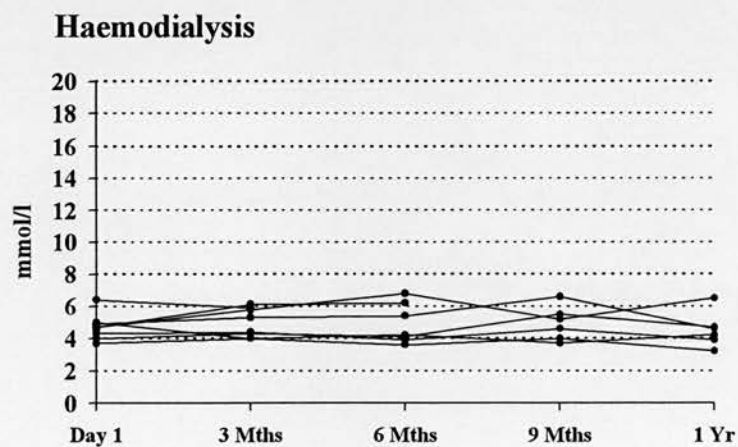
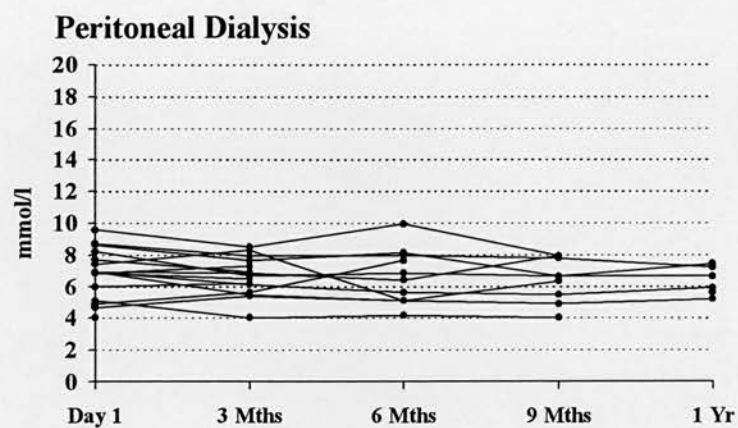


Figure 10.15 Fasting cholesterol during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

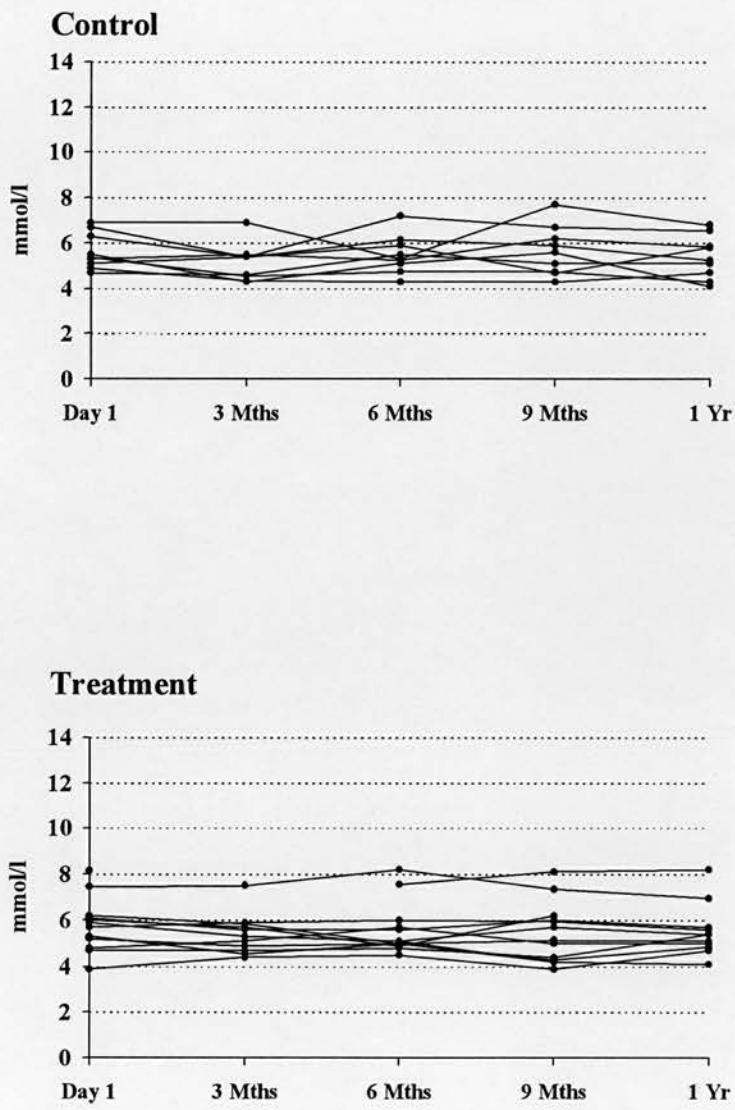


Figure 10.16 Fasting cholesterol during the first year of study in the transplant control and treatment groups. There was no difference between the change in cholesterol in the two groups.

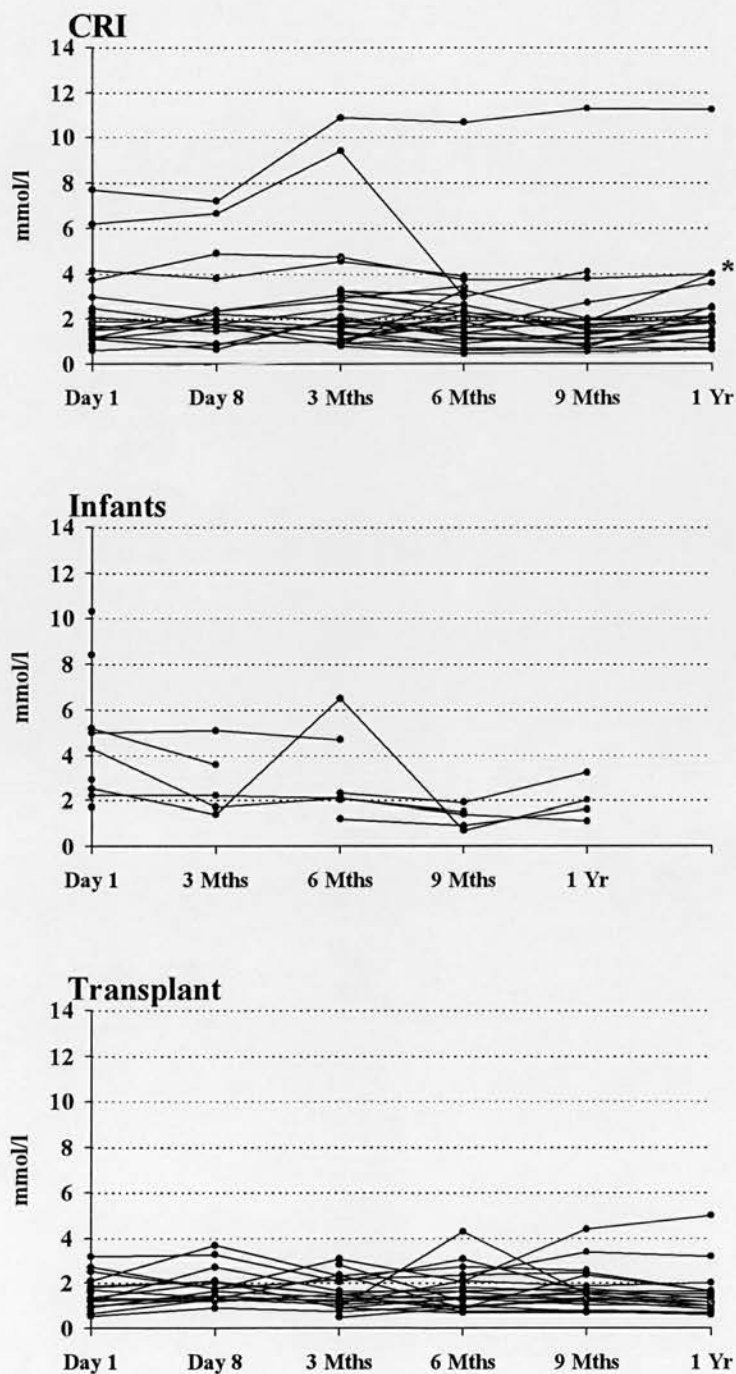


Figure 10.18 Fasting triglyceride during rhGH treatment in the CRI, infant and transplanted groups. * Triglyceride levels increased during rhGH treatment in the CRI group, $p = 0.004$, ANOVA.

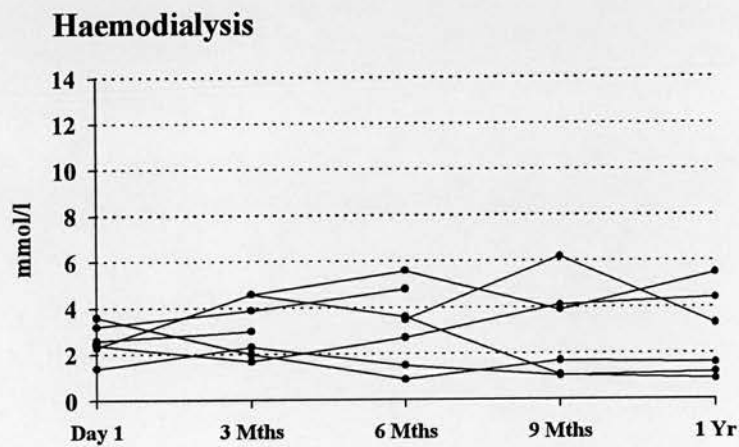
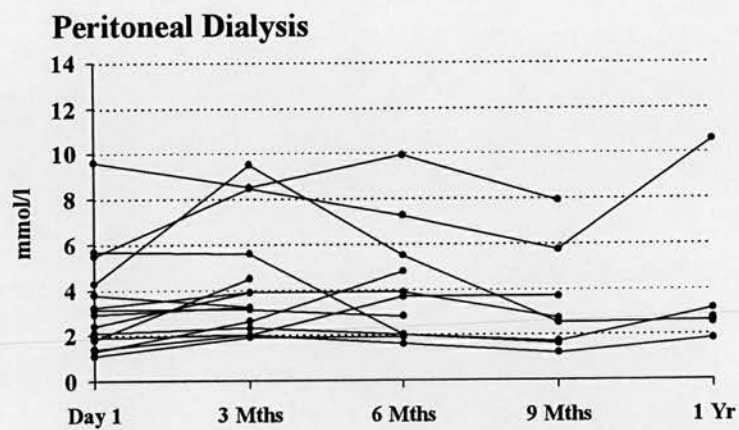


Figure 10.19 Fasting triglyceride during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

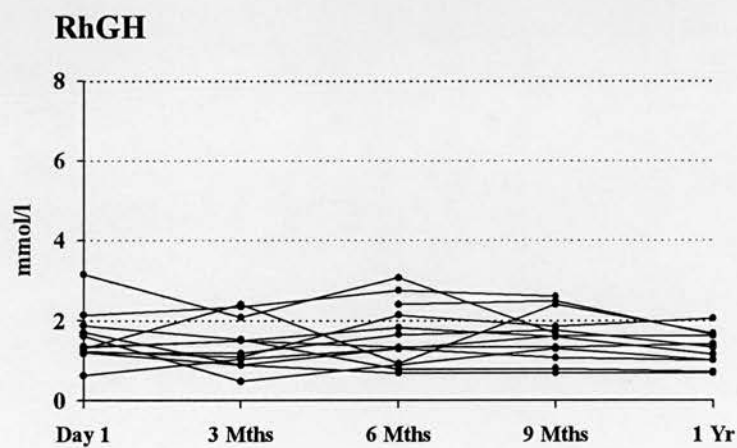
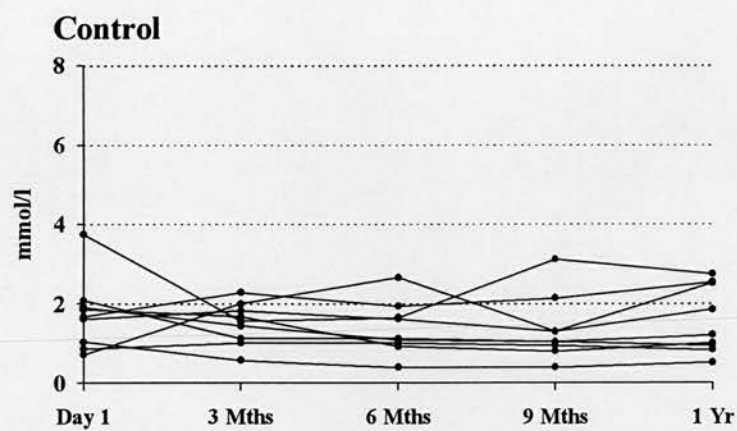


Figure 10.20 Fasting triglyceride during the first year of study in the transplant control and treatment groups.

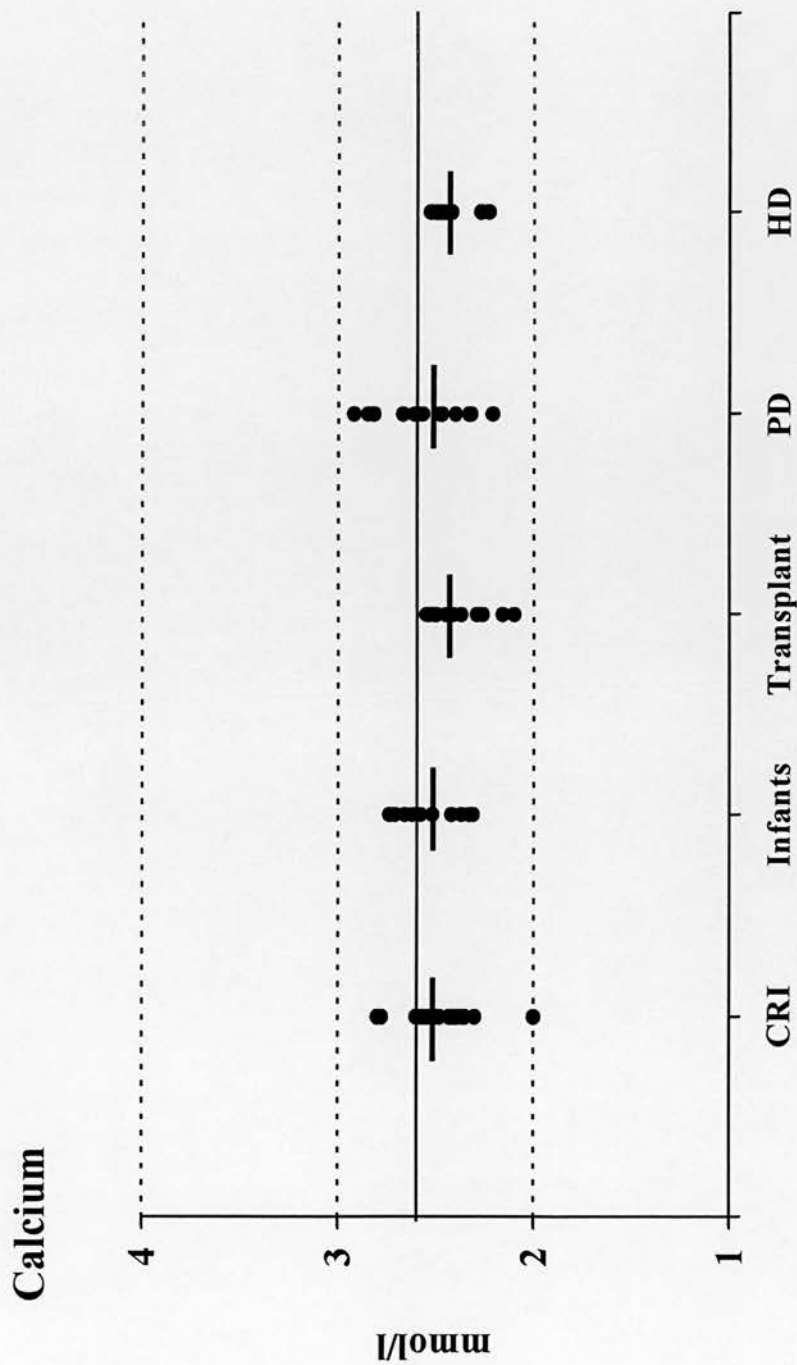


Figure 10.21 Plasma calcium at the start of the study in the CRI, infant, transplanted, peritoneal dialysis (PD) and haemodialysis (HD) groups. The upper limit of normal is indicated by the solid line.

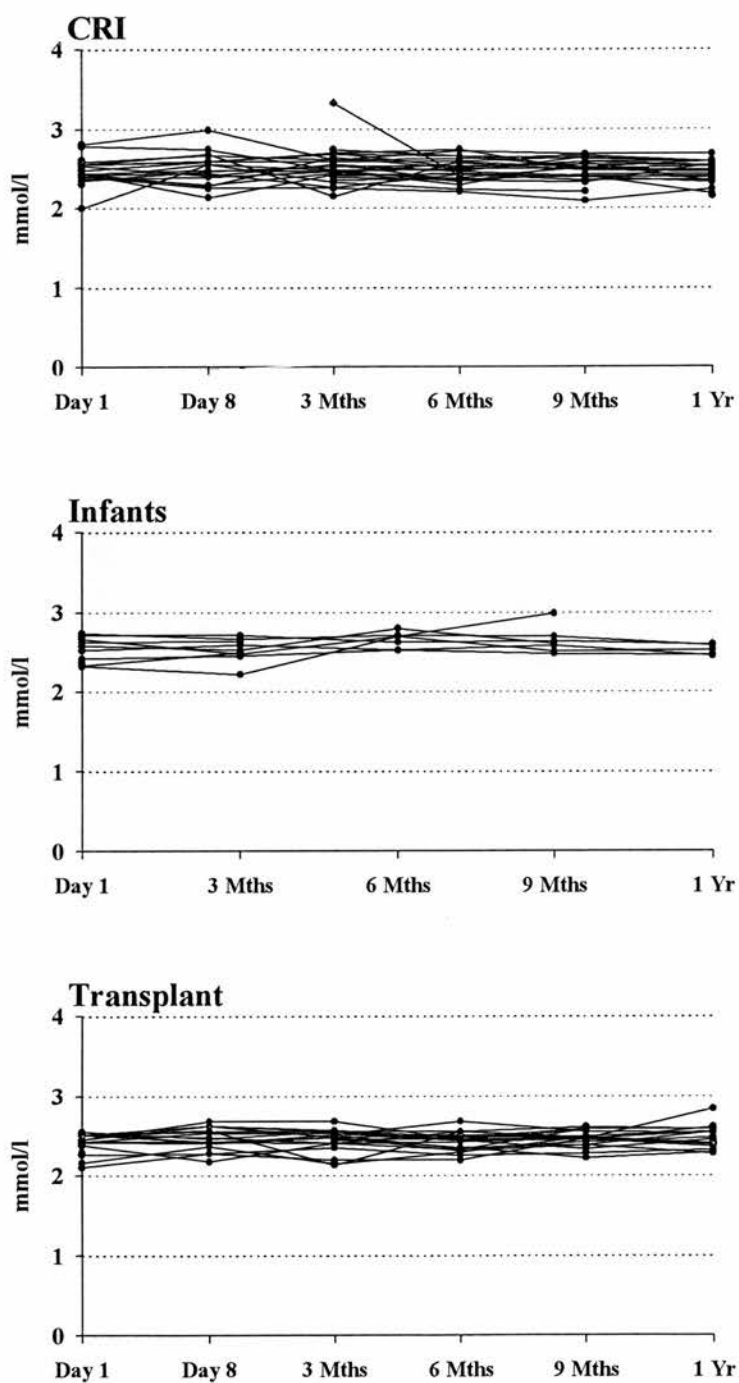


Figure 10.22 Plasma calcium during rhGH treatment in the CRI, infant and transplanted groups.

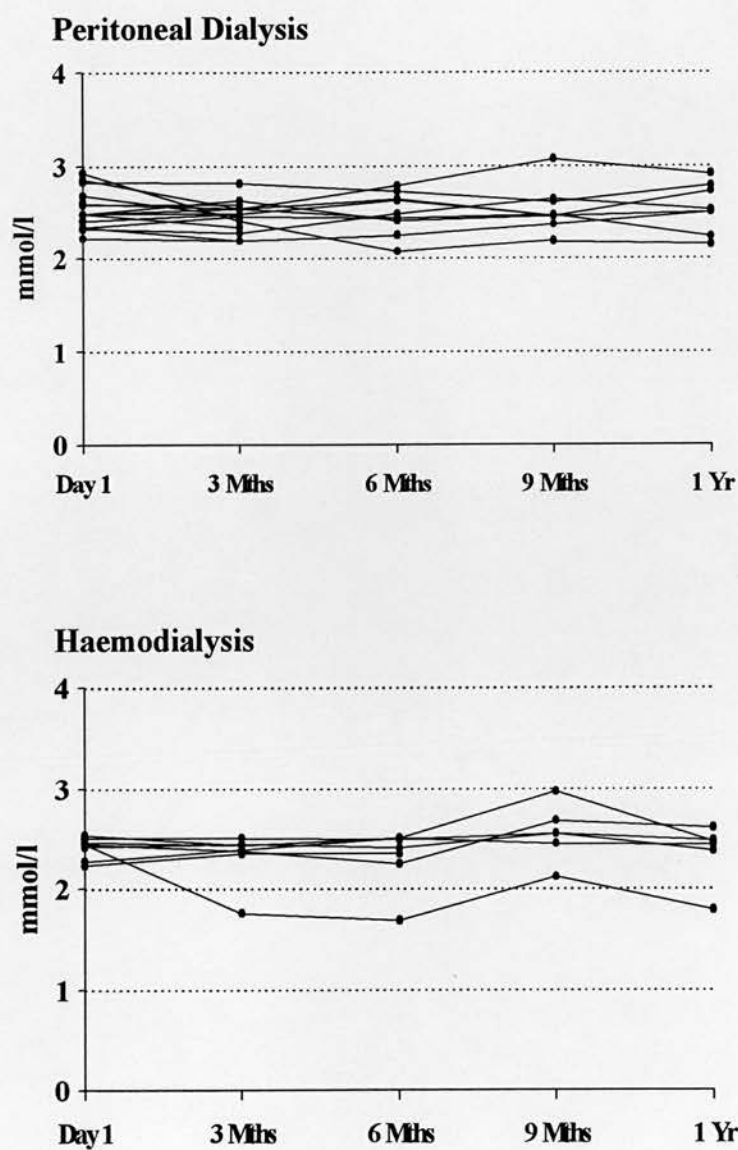


Figure 10.23 Plasma calcium during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

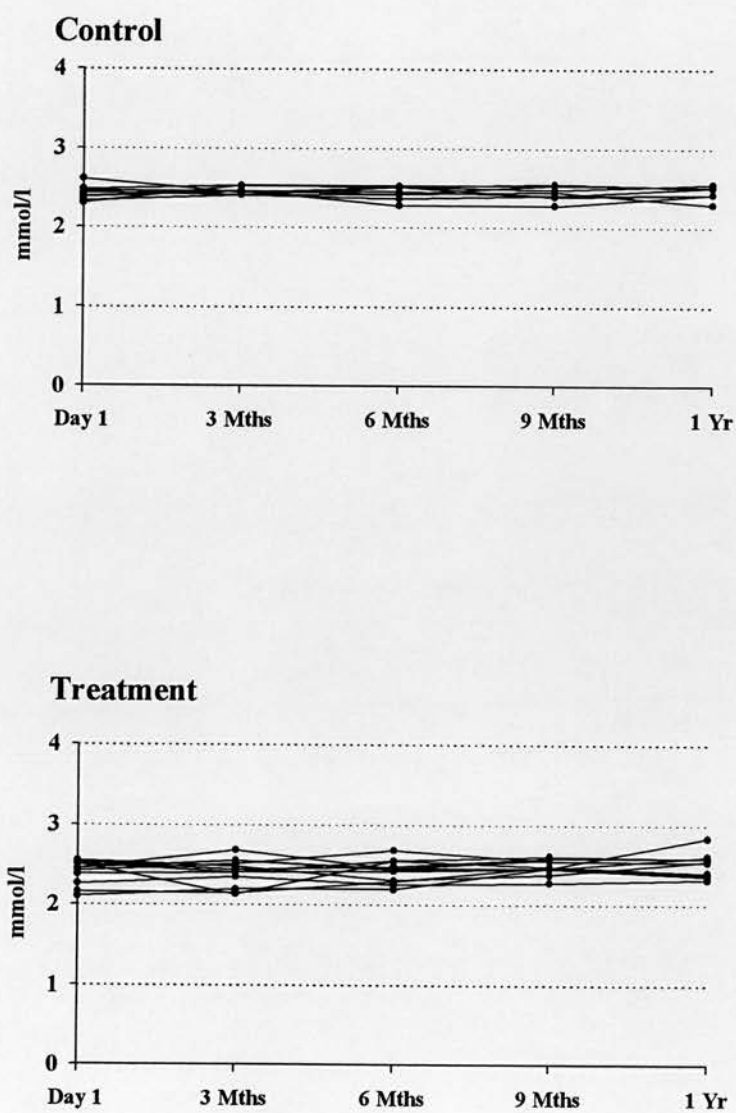


Figure 10.24 Plasma calcium during the first year of study in the transplant control and treatment groups.

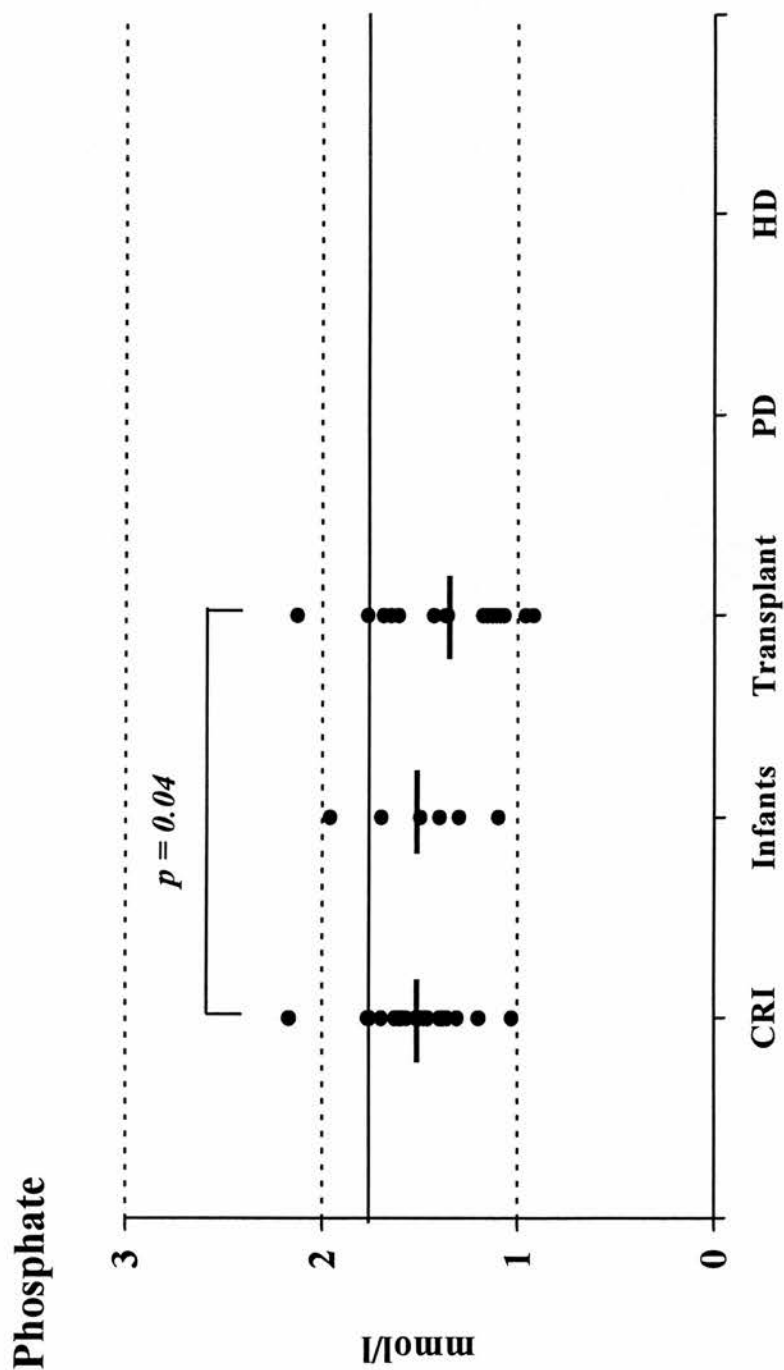


Figure 10.25 Plasma phosphate at the start of the study in the CRI, infant and transplanted groups. Differences between the groups are as shown. The upper limit of normal is indicated by the solid line.

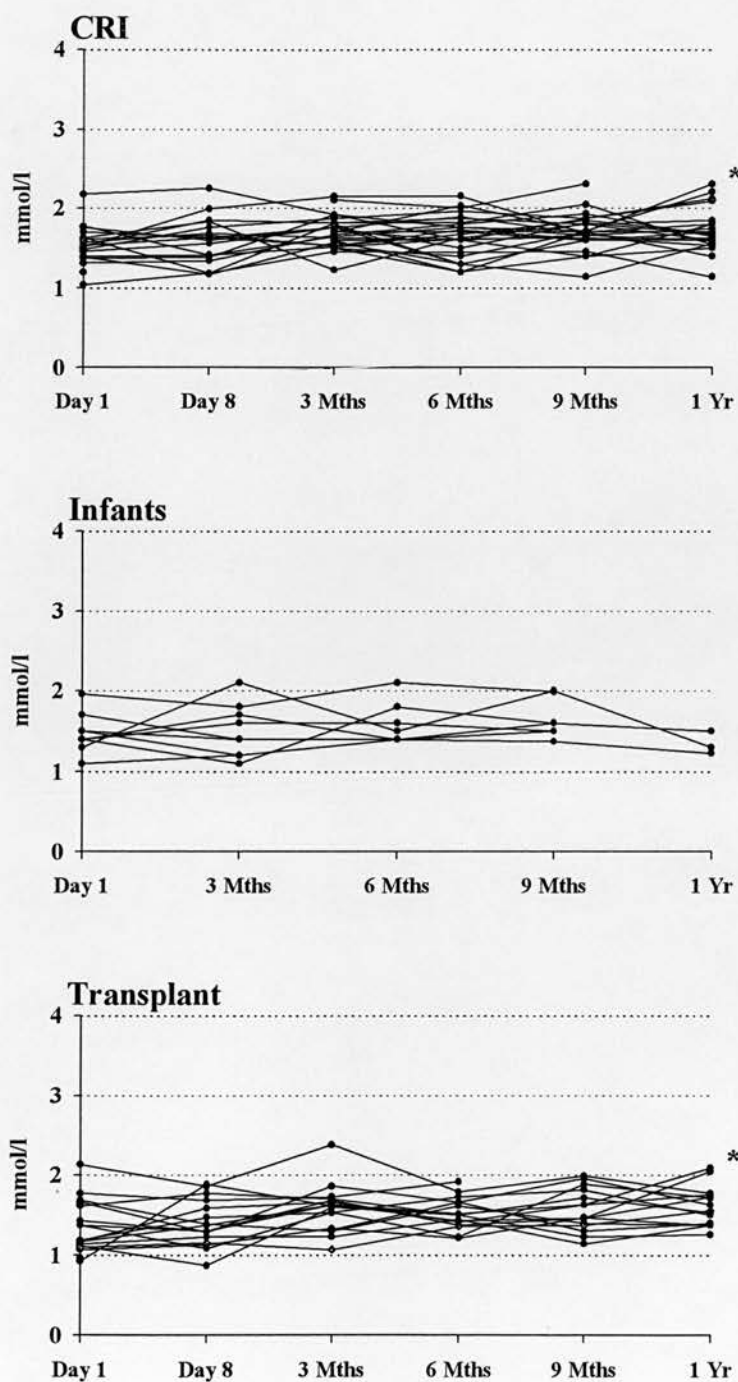


Figure 10.26 Plasma phosphate during rhGH treatment in the CRI, infant and transplanted groups. * Phosphate increased during treatment in the CRI ($p < 0.001$) and transplant ($p < 0.001$) groups, ANOVA.

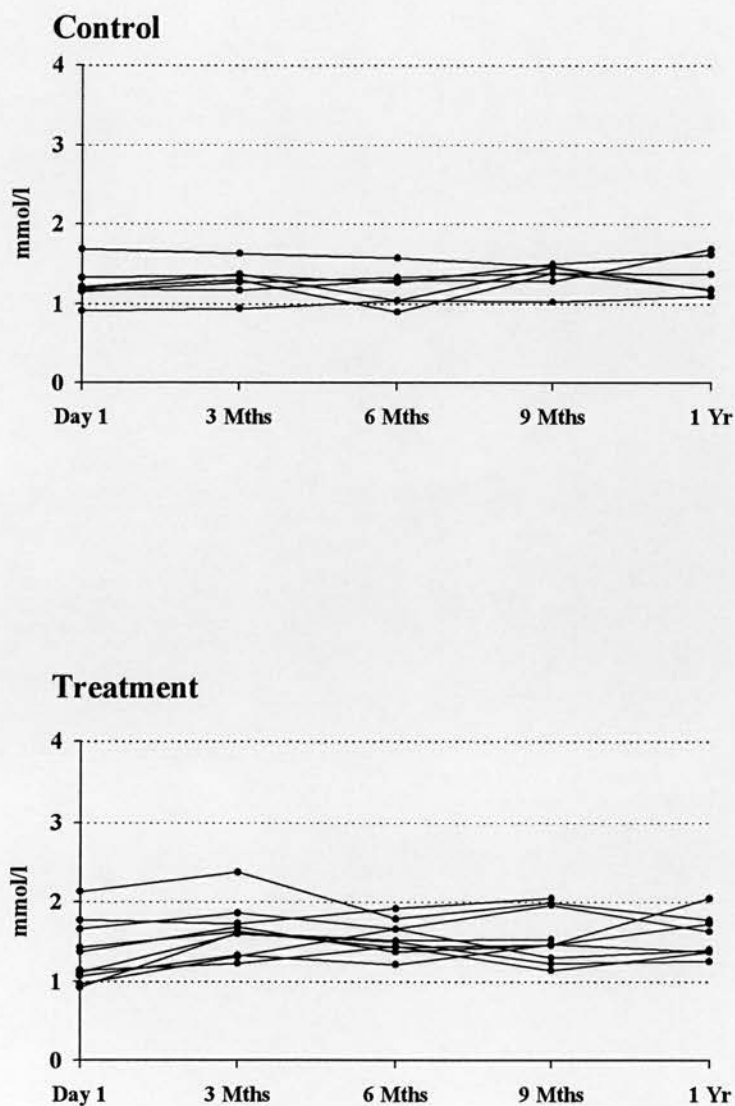


Figure 10.27 Plasma phosphate during the first year of study in the transplant control and treatment groups. * Phosphate increased in the treatment group ($p = 0.02$, ANOVA) but not the control group ($p = 0.41$, ANOVA).

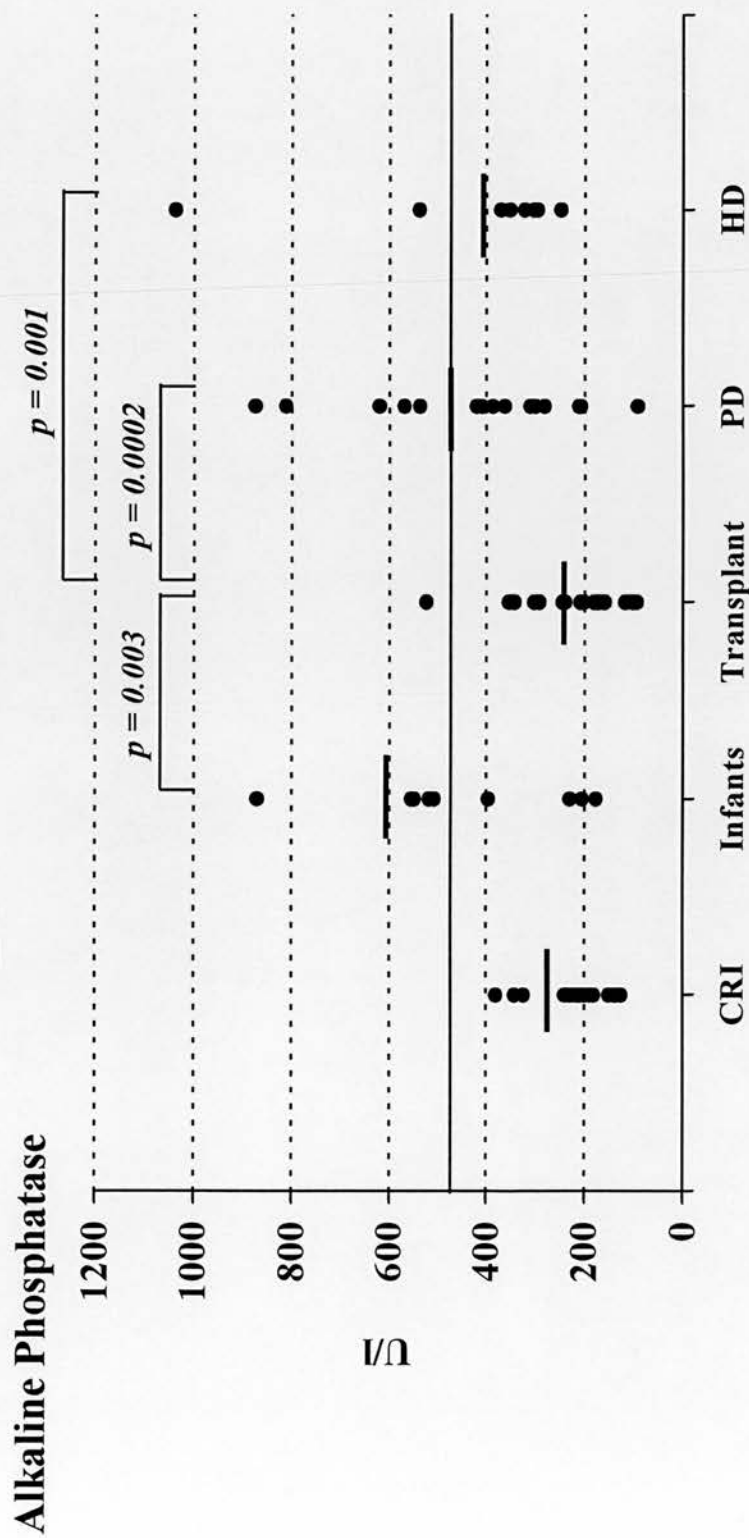


Figure 10.28 Alkaline phosphatase at the start of the study in the CRI, infant, transplant, peritoneal dialysis (PD) and haemodialysis (HD) groups. Individual and median values are shown. Differences between the groups are as shown. The upper limit of normal is indicated by the solid line.

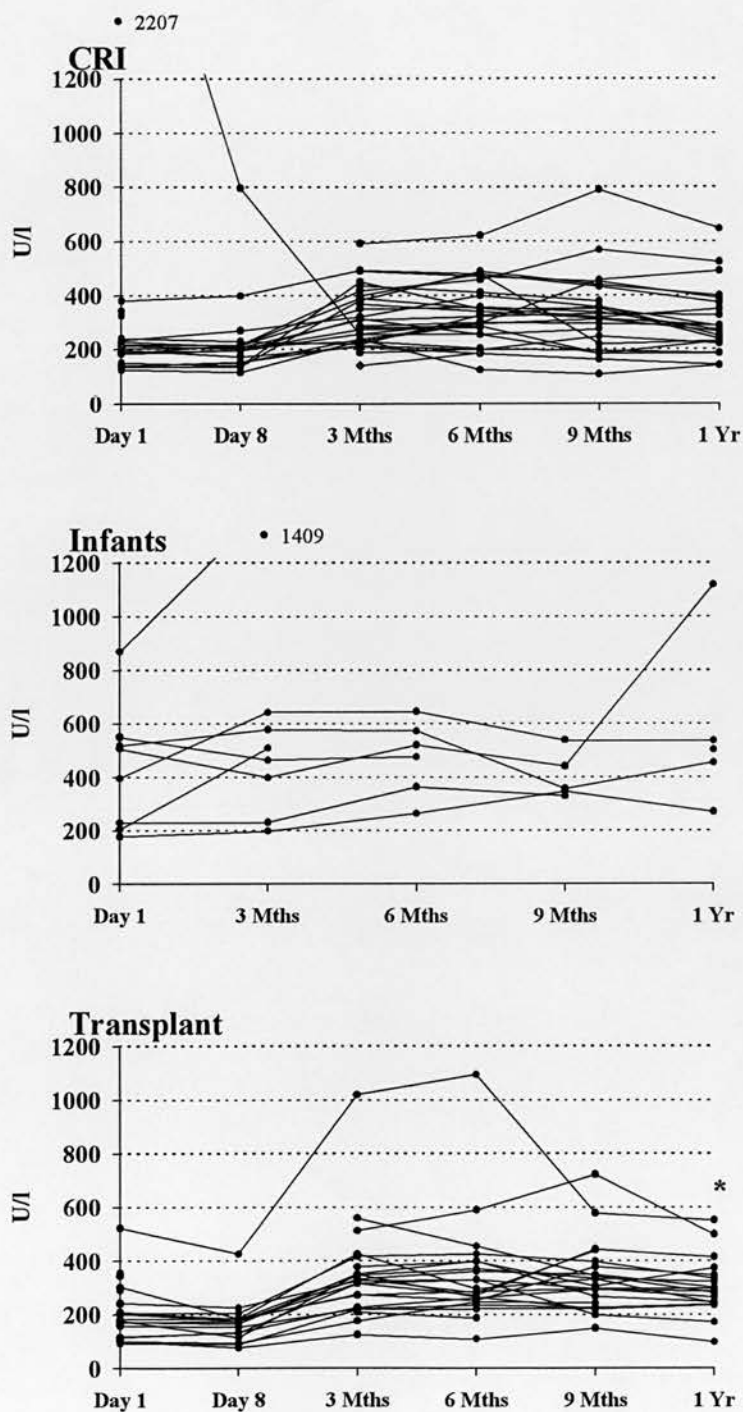


Figure 10.29 Alkaline phosphatase (ALP) during rhGH treatment in the CRI, infant and transplant groups. * An increase in ALP was seen in the transplant group, ($p < 0.0001$, ANOVA).

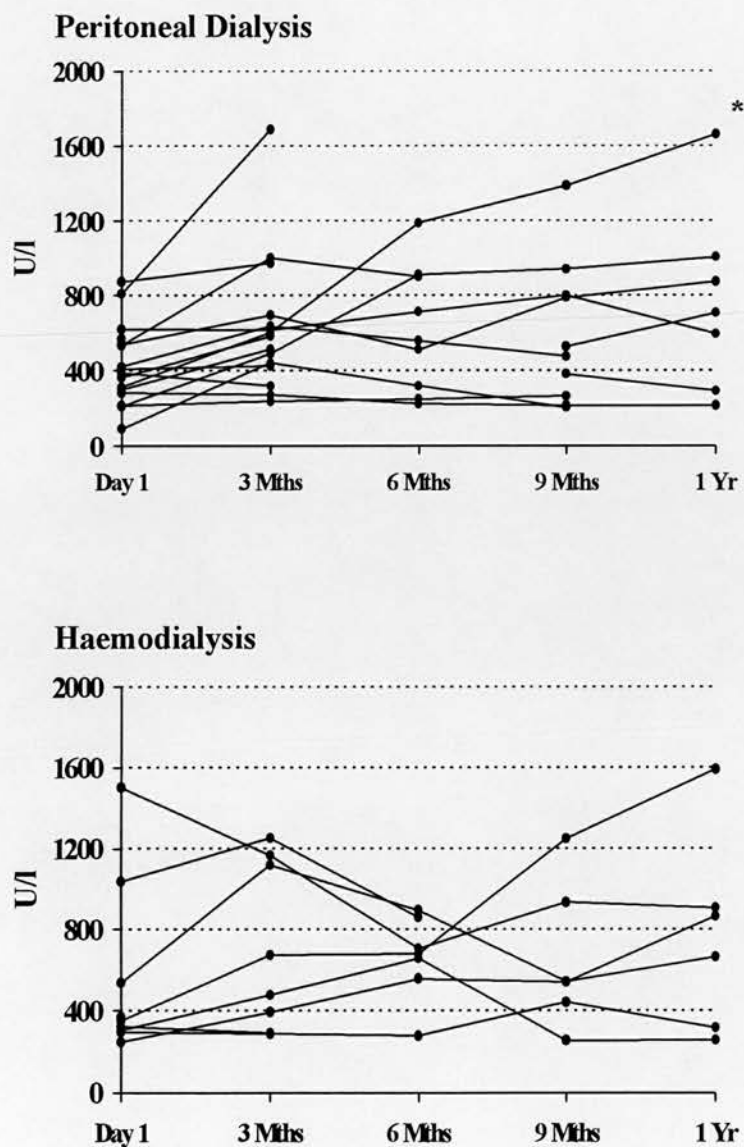


Figure 10.30 Alkaline phosphatase (ALP) during rhGH treatment in the peritoneal dialysis (PD) and haemodialysis groups. * ALP increased during treatment in the PD group ($p = 0.03$, ANOVA).

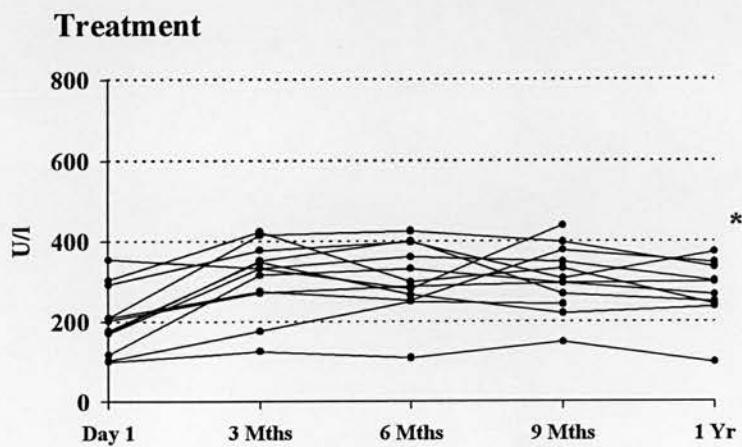
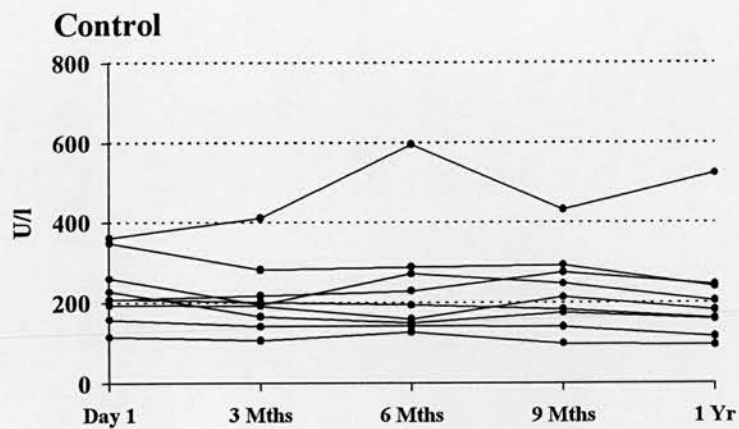


Figure 10.31 Alkaline phosphatase (ALP) during the first year of study in the transplant control and treatment groups. * ALP increased in the treatment group ($p < 0.0001$, ANOVA), but not the control group ($p = 0.55$, ANOVA).

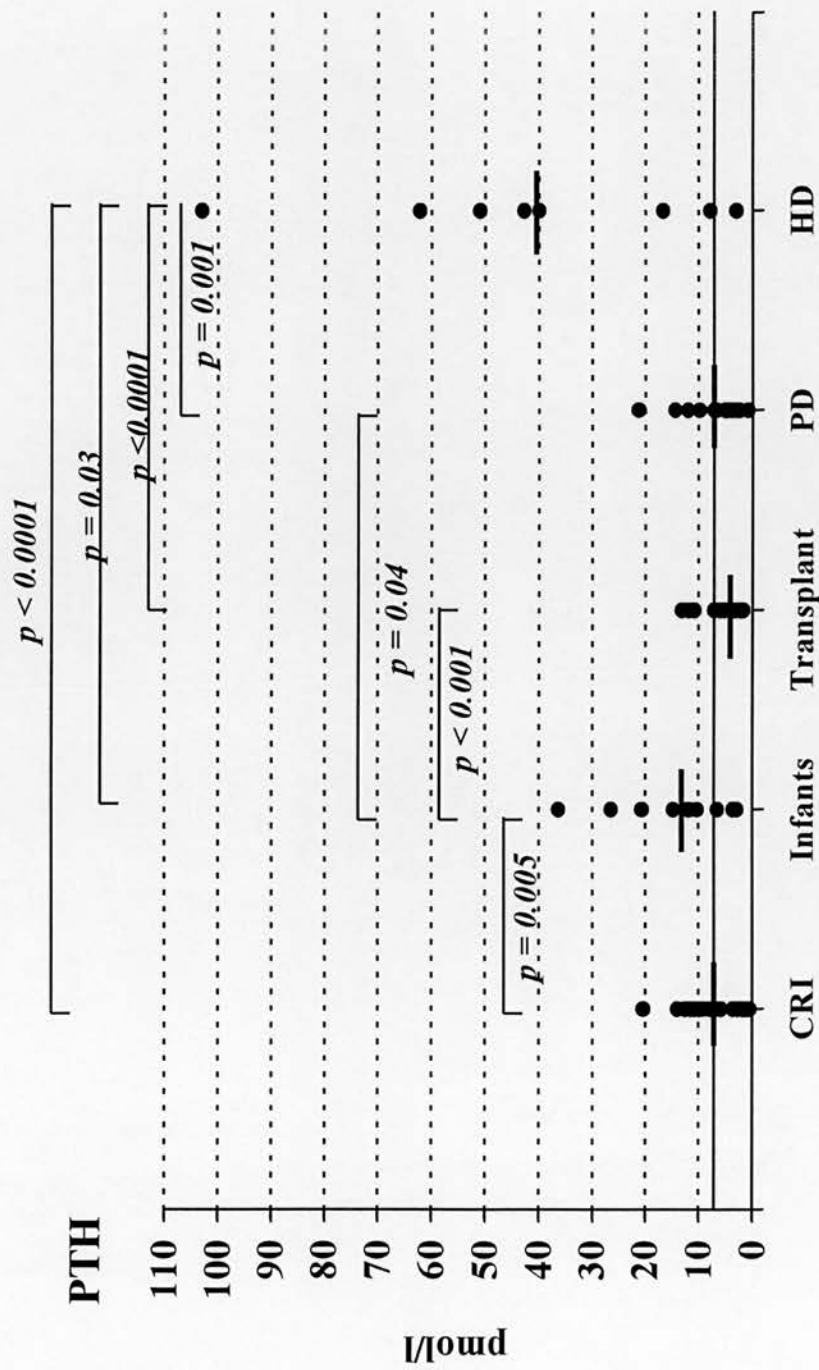


Figure 10.32 Parathyroid hormone (PTH) at the start of the study in the CRI, infant, transplanted, peritoneal dialysis (PD) and haemodialysis (HD) groups. Individual and median values are shown. The upper limit of normal is indicated by the solid line.

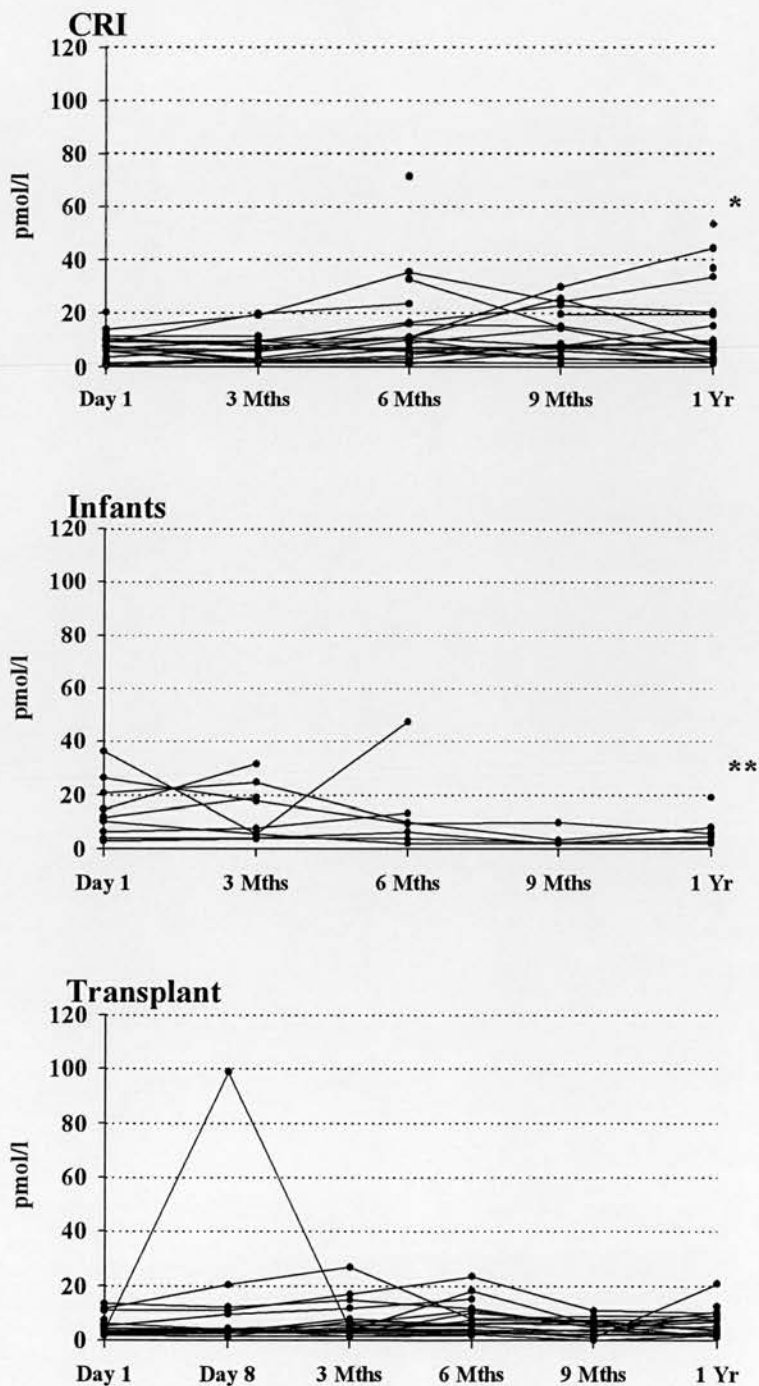


Figure 10.33 Parathyroid hormone (PTH) during rhGH treatment in the CRI, infant and transplanted groups. * PTH increased during treatment in the CRI group ($p = 0.12$, ANOVA), and ** decreased in the infant group, ($p = 0.03$, ANOVA).

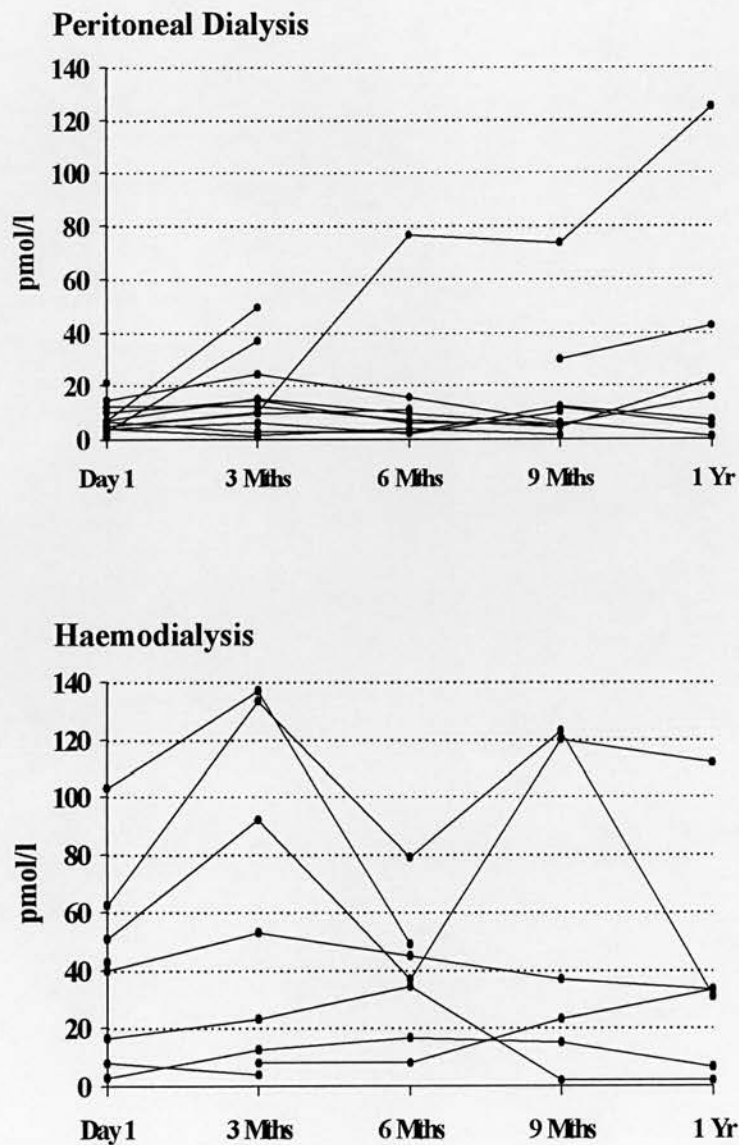


Figure 10.34 Parathyroid hormone during rhGH treatment in the peritoneal dialysis and haemodialysis groups.

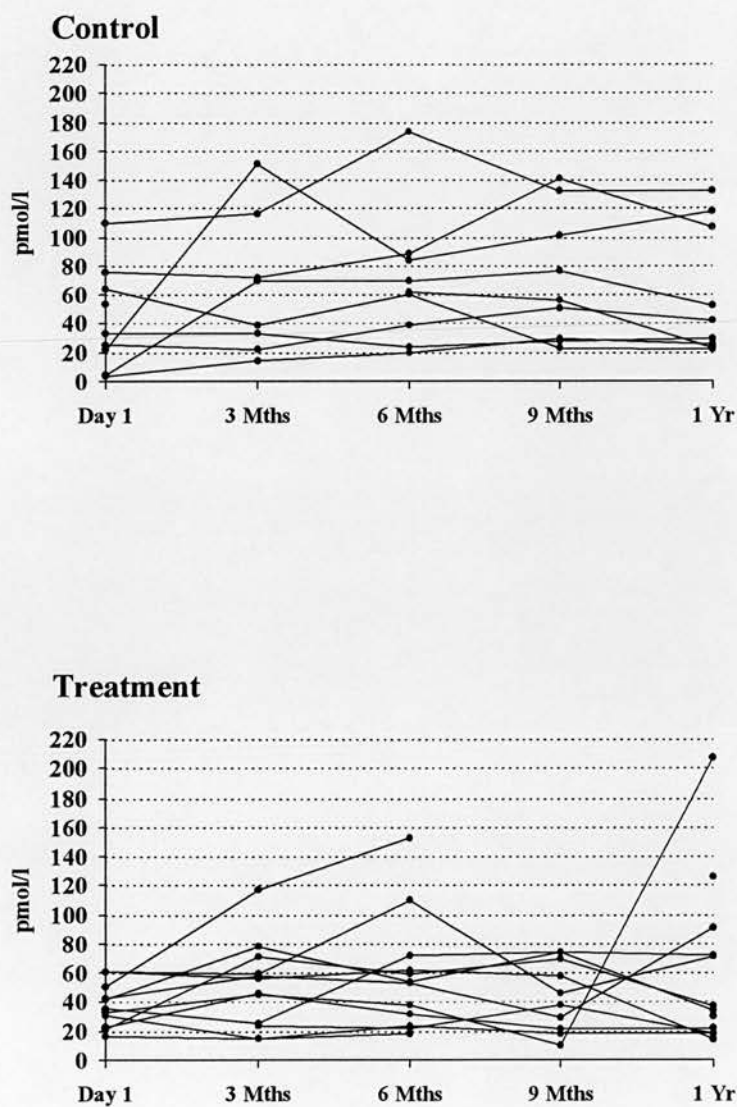


Figure 10.35 Parathyroid hormone (PTH) during the first year of study in the transplant control and treatment groups. PTH increased in the treatment group ($p = 0.05$, ANOVA), but not in the control group ($p = 0.35$, ANOVA).

Chapter 11: Concluding Remarks

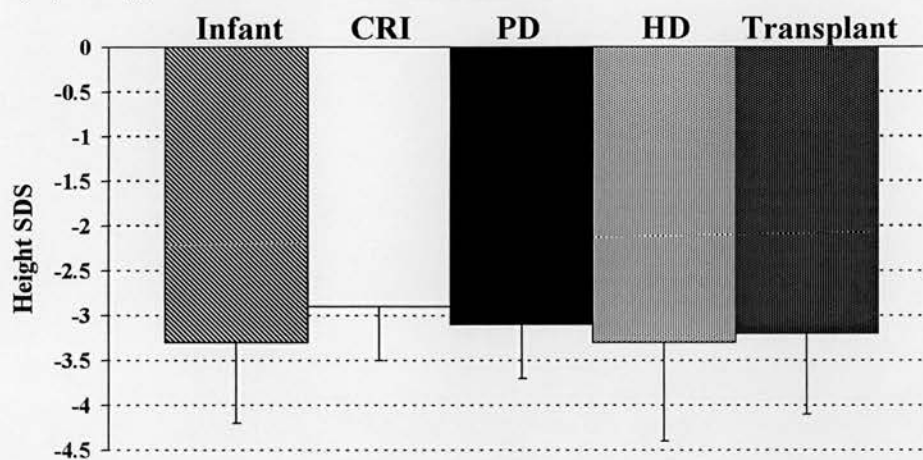
Short stature is a problem in CRF and in children with renal transplants. All of the groups of children studied had significant short stature (Figure 11.1A). The greatest increase in height SDS was seen in the infant group, with the poorest response being seen in the dialysis groups (Figure 11.1B). Increase in height SDS was greater the younger the child in the infant, CRI and transplanted groups. In the transplanted group, Δ height SDS was also negatively correlated with the dose of prednisolone.

Height velocity increased in all groups of patients during rhGH treatment (Figures 11.2 A and B). The greatest increase in HV was seen in the children who were growing most slowly in the infant, CRI and combined dialysis groups.

Growth hormone was well tolerated in the majority of patients. However two serious adverse effects occurred during the trial. One child developed benign intracranial hypertension from which she made a full recovery. Another child developed glucose intolerance requiring withdrawal from the study. A further child developed biochemical evidence of renal osteodystrophy, and several of the transplanted children had presumed episodes of rejection, however for these children it is not possible to be certain if growth hormone treatment was responsible.

In the short to medium term, rhGH appears to be an effective treatment for short stature of CRF and renal transplantation. Growth hormone treatment however is not without side-effects, albeit rare side-effects, and due consideration needs to be taken before commencing treatment, particularly in the transplanted patient. The potential problems need to be explained to the family, and the development of symptoms such as headaches needs to be taken seriously. The effect of rhGH treatment on final height is not yet clear, but final height data are awaited with anticipation.

(A) Height SDS Before rhGH Treatment



(B) Δ Height SDS During rhGH Treatment

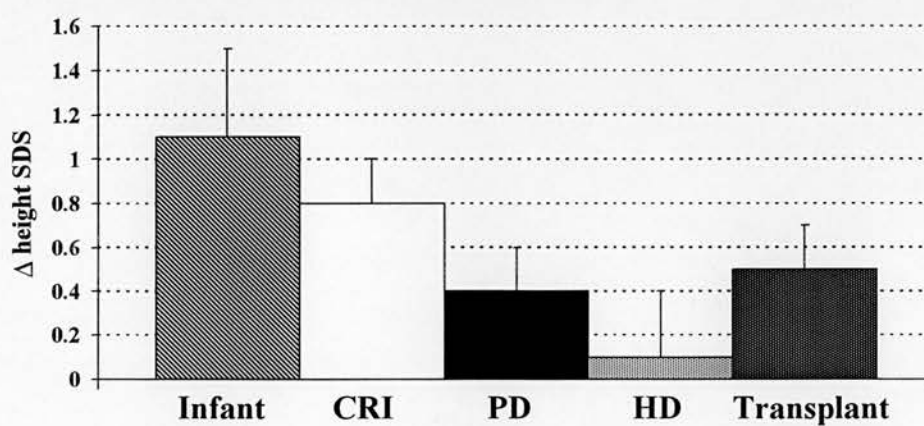


Figure 11.1 (A) Height SDS at the start of the study in the infant, CRI, PD, HD and transplanted groups. (B) Increase in height SDS during the first year of rhGH treatment in the same groups of children.

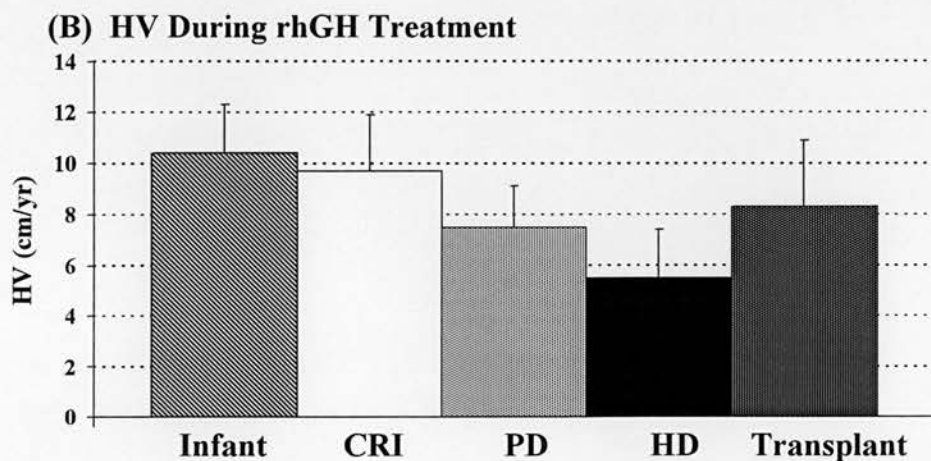
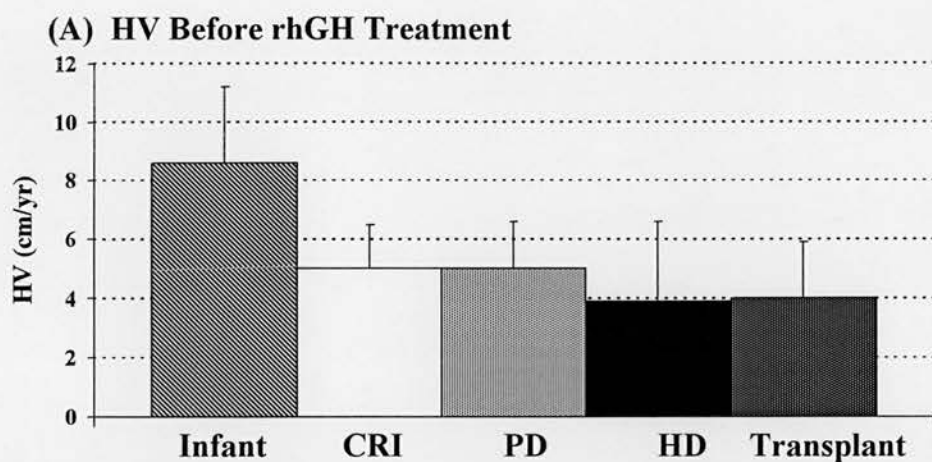


Figure 11.2 (A) Height velocity in the year before the study in the infant, CRI, PD, HD and transplanted groups. (B) Height velocity during the first year of rhGH treatment in the same groups of children.

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Original article

Differential effects of recombinant human growth hormone on glomerular filtration rate and renal plasma flow in chronic renal failure

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Abstract. In normal subjects recombinant human growth hormone (rhGH) increases glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) through the action of insulin-like growth factor-I (IGF-I). We have measured clearance of inulin and para-aminohippuric acid in 18 children with chronic renal failure (CRF) during their 1st year of rhGH treatment to look at the immediate (first 3 h), short-term (1 week) and long-term (1 year) effects of treatment. On day 1 mean (range) age was 9.1 (4.9–13.9) years, GFR 19 (9–58) and ERPF 77 (34–271) ml/min per 1.73 m². During treatment height velocity increased from 4.5 (1.7–6.5) to 9.5 (4.8–12.7) cm/year ($P < 0.0001$). Two children required dialysis after 0.75 years and 1 child was electively transplanted after 0.5 years. There were no other serious adverse events. GFR and ERPF were unchanged in the 3 h following rhGH. GFR remained constant on day 8, 22 (6–56) and after 1 year, 20 (9–59) ml/min per 1.73 m². ERPF increased to 96 (33–276) ml/min per 1.73 m² on day 8 ($P = 0.005$), and remained elevated, but not significantly so, at 99 (24–428) ml/min per 1.73 m² at 1 year. Fasting IGF-I increased from 147 (46–315) ng/ml to 291 (61–673) by day 8 ($P < 0.003$), and to 341 (101–786) ng/ml at 1 year. There was no correlation between the change in IGF-I and renal function. Blood pressure, albumin excretion and dietary protein intake were unchanged by treatment. The significance of increased ERPF after 1 week of rhGH in CRF is unclear, but long-term follow-up of renal function is indicated.

Key words: Recombinant human growth hormone – Insulin-like growth factor-I – Chronic renal failure – Glomerular filtration rate – Effective renal plasma flow

Introduction

Several studies have shown that recombinant human growth hormone (rhGH) is effective in the treatment of short stature of chronic renal failure (CRF) [1–3]. What is not so clear at present is the safety of its use. Of particular concern is the effect on renal function: rhGH, when given experimentally to adults with normal renal function, increases glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). This effect occurs after several hours, at a time when growth hormone (GH) levels have returned to normal but levels of the GH mediator, insulin-like growth factor-I (IGF-I), are elevated [4–7]. Indeed, administration of recombinant human IGF-I (rhIGF-I) increases GFR and ERPF [8,9]. rhGH increases IGF-I levels in CRF [1], but no changes in GFR have been found, either in adults given rhGH experimentally [10] or in children in therapeutic trials of the use of rhGH in short stature of renal disease [11–13].

The effects of rhGH on ERPF in CRF in man have not been reported. Recently however rhIGF-I has been shown to increase both GFR and ERPF in adults with moderate CRF [14]. If hyperfiltration is a mechanism for progression of CRF in man, as appears to be the case in small mammals [15], then rhGH treatment in children with CRF may lead to a deterioration in renal function. We have therefore looked at the effects of 1 week and 1 year of rhGH treatment on renal function and levels of IGF-I in children with CRF. In addition, we have looked at the immediate effects of rhGH in the 3–4 h following a subcutaneous injection.

Patients and methods

Eighteen children with CRF have been studied. All were regularly attending CRF clinics. At the start of the study, mean age was 9.1 (4.9–13.9) years, mean GFR, as measured by inulin clearance, 19 (9–58) ml/min per 1.73 m² and mean height standard deviation score –3.0 (–4.8 to –1.8) using the Tanner and Whitehouse standards for British children [16]. Aetiology of renal disease was congenital structural problems in 15 children and focal segmental glomerulosclerosis (FSGS) in 3. All were prepubertal, except 1 boy who pro-

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gressed from pubertal stage II to III during the year of study. rhGH (Genotropin, Pharmacia, Stockholm) was given for 1 year, at a dose of 1 IU/kg per week, as a daily subcutaneous injection every evening. Written informed consent was obtained in all cases, and ethical approval obtained from the participating hospitals.

The children were examined on day 1 and day 8, then at 3-monthly intervals. Height, weight and blood pressure were recorded, blood drawn (approximately 12 h after the previous dose of rhGH) for estimation of GH and IGF-I, a full blood count and biochemical profile, including plasma creatinine, urea and electrolytes. On each day an early-morning urine sample was obtained for measurement of microalbumin. Three-day dietary assessments were made 6 monthly (2 children received overnight nasogastric feeds).

GFR and ERPF were estimated by clearance of inulin (Inutest 25%, Laevosan-Gesellschaft, Linz/Donau) and para-aminohippuric acid (PAH) (aminohippurate sodium 20%, Merck, Sharp and Dohme, West Point, USA) respectively, using a standard clearance with urine collection technique, described below. This procedure was carried out on day 1, day 8 and at 1 year; on days 1 and 8 rhGH was given at midday rather than in the evening. On day 1, eight urine collections were made; the first three to establish baseline clearance. Immediately the third collection was complete, the first subcutaneous injection of rhGH was given and the urine collections continued without interruption. On day 7 rhGH was given in the evening as usual, then exactly the same procedure as above was carried out the following morning (day 8), before and after the eighth injection of rhGH. Baseline clearances (pre) were taken as the average of the first three collections and compared with the average of the last three collections (nos. 6–8) (post) to look for an immediate effect of rhGH. At 1 year, four 40-min urine collections were carried out, 12 h after the last injection of rhGH. Baseline clearances on day 1, day 8 and after a year were compared, to look at the short- and long-term effects of rhGH. Four of the younger children had baseline clearance tests only on each of the 3 days, four 40-min urine collections before rhGH on day 1, and at least 12 h after the previous dose on day 8 and at 1 year.

The procedure was carried out after an overnight fast. A cannula was inserted in each arm, a fasting blood sample withdrawn, then a bolus of 50 mg/kg inulin and 5 mg/kg PAH in 50 ml of normal saline given over 3–4 min. This was followed by infusion of a maintenance solution of inulin and PAH in 0.45% saline, calculated to maintain a plasma inulin level of approximately 250 mg/l ($\text{mg inulin/min} = 250/1.000 \times \text{GFR}$) and 25 mg/l PAH ($\text{mg PAH/min} = \text{GFR}/0.2 \times 25/1.000$). The maintenance solution was infused at 100 ml/h, by constant rate infusion pump (IMED Volumetric Pump 960, IMED, Alington, Bedford, UK). Diuresis was induced by encouraging the child to drink water, 20 ml/kg for the 1st h, then 10 ml/kg per hour thereafter. As the study lasted for several hours, the child was allowed to eat small

non-protein, non-fructose-containing snacks. After 1 h, steady state was assumed and the bladder emptied. Eight 40-min urine collections (collections 1–8) were made with the child being encouraged to completely empty the bladder on each occasion. A sample of blood was drawn, from the second cannula, at the midpoint of each collection for inulin, PAH and GH. IGF-I was measured during collections 3 and 8. rhGH (0.14 IU/kg) was given by subcutaneous injection on completion of the third collection.

Inulin was measured spectrophotometrically using a resorcinol method [17]. PAH was determined by the Bratton Marshall reaction [18], creatinine by an enzymatic method using an RA1000 autoanalyser (Technicon Instruments, Basingstoke, UK) and IGF-I by radioimmunoassay after acid-ethanol extraction. It is recognised that this method of extraction does not completely remove IGF-binding proteins [19], which can then interfere in the assay. The extent of interference appears to depend on the particular antibody being used. In our assay, acid chromatography (AC), when compared with acid-ethanol (AE) extraction, gave comparable results in CRF ($r = 0.979$, $P < 0.0001$) with AE giving slightly lower results [$\text{AE} = (0.906 \times \text{AC}) - 0.049$]. GH was measured by immunoradiometric assay. Urinary microalbumin was measured by an immunoturbidimetric method (Technicon method, Bayer Diagnostics, UK). Urinary creatinine was measured enzymatically on the same urine samples, and the results expressed as the albumin/creatinine ratio.

Statistical analysis was performed using a paired Student's *t*-test (and confirmed using a Wilcoxon matched pairs signed rank sum test). A *P* value of <0.05 was taken as indicating a statistically significant difference. Analysis of repeated measurements at different time points in the same individual was by two-way analysis of variance. Correlation was performed by Pearson's correlation and multiple regression analysis. Results are given as mean (range) unless otherwise indicated.

Results

Of the 18 children, 15 completed 1 year of treatment; 2 required dialysis after 9 months of treatment and the other received a pre-emptive transplant after 6 months. Data from these children, whilst they were in the trial, have been included and their height velocities on treatment annualised. There were no other serious adverse events. Height velocity increased from 4.5 (1.7–6.5) cm/year in the year before treatment to 9.5 (4.8–12.7) cm/year whilst on treatment ($P < 0.0001$).

Table 1. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), insulin-like growth factor-I (IGF-I) and growth hormone (GH) before and after recombinant human GH (rhGH) on days 1 and 8^{a, b}

	Day 1		Day 8	
	pre	post	pre	post
<i>n</i> = 14				
GFR (ml/min per 1.73 m ²)	22 (17) (9–60)	21 (16) (10–60)	23 (14) (6–58)	25 (15) (7–53)
ERPF (ml/min per 1.73 m ²)	81 (64) (35–281)	80 (55) (35–247)	99 (67)* (42–272)	104 (72)* (38–272)
IGF-I (ng/ml)	145 (67) (51–255)	129 (61) (32–222)	238 (141)*** (59–546)	236 (141)*** (89–590)
GH (mU/l)	12.2 (12.0) (0.5–35.8)	105.9 (35.3)** (33–166.8)	14.0 (11.2) (1.8–34.7)	163.6 (75.9)** (80–291)

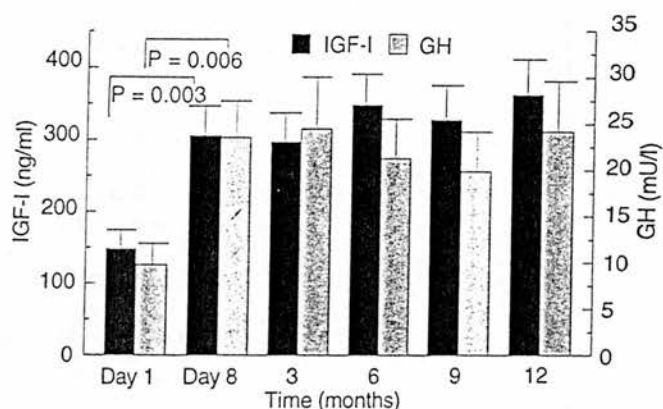
^a $P < 0.03$ day 1 vs. day 8; ^{**} $P < 0.001$ post vs. pre; ^{***} $P < 0.005$ day 1 vs. day 8

Mean (SD) and range

^b Pre rhGH IGF-I and GH values were taken 20 min before injection of rhGH; post rhGH values were taken 3 h after injection

Table 2. GFR, ERPF, filtration fraction and blood pressure (BP) during the year of rhGH treatment^a

	All patients after 1 week		Patients completing study	
	Day 1	Day 8	Day 1	1 Year
	(n = 18)	(n = 18)	(n = 15)	(n = 15)
Baseline GFR	19 (13) (9–58)	22 (13) (6–56)	19 (14) (9–58)	20 (16) (9–59)
Baseline ERPF	77 (54) (34–271)	96 (62)* (33–276)	78 (58) (34–271)	99 (111) (24–428)
Filtration fraction	0.26 (0.07) (0.15–0.40)	0.25 (0.09) (0.11–0.41)	0.25 (0.07) (0.15–0.40)	0.24 (0.08) (0.11–0.38)
Systolic BP	97 (6) (90–110)	97 (6) (87–109)	97 (6) (90–110)	98 (11) (75–120)
Diastolic BP	58 (8) (40–70)	59 (7) (45–70)	59 (8) (40–70)	64 (6) (40–85)

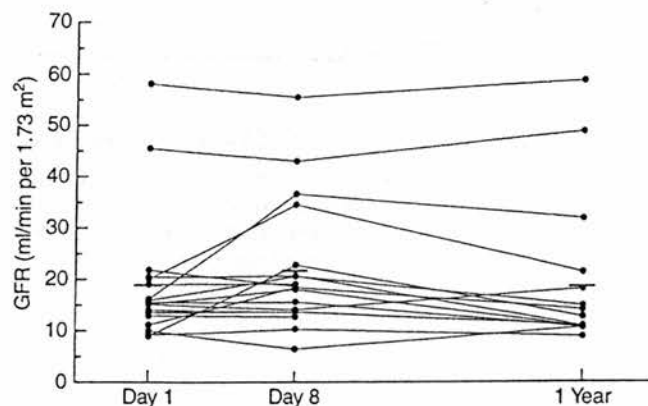
* $P = 0.005$ day 1 vs. day 8^a Mean (SD) and range**Fig. 1.** Mean (SEM) fasting insulin-like growth factor-I (IGF-I) and growth hormone (GH) during the year of study

Immediate effects of rhGH

Fourteen children had clearance studies before and after a subcutaneous injection of rhGH on days 1 and 8. Results are given in Table 1. GH increased approximately ten-fold in the 3–4 h following rhGH on both days 1 and 8. There was no change in IGF-I, GFR or ERPF during this time on either day.

Results at 1 week

Eighteen patients had baseline clearances before and after 1 week of rhGH (Table 2). Mean fasting GH and IGF-I were elevated at 1 week (Fig. 1). There was no change in mean GFR (Fig. 2) but there was a significant increase of approximately 25% in mean ERPF (Fig. 3). In individual patients the increase in ERPF was in the order of 6%–30% in 8, and between 32%–131% in a further 6 patients. Three patients showed a decline in ERPF (of 3%, 4% and 22%) and in 1 patient there was no change. There was no relationship between day 1 GFR or ERPF and the change in ERPF. Four patients showed an increase in GFR in addition

**Fig. 2.** Clearance of inulin [glomerular filtration rate (GFR)] in individual patients on day 1, day 8 and after 1 year of recombinant human growth hormone (rhGH) treatment. The mean value on each day is shown

to an increase in ERPF. This subgroup was not distinguished by baseline renal function, aetiology of renal disease, basal IGF-I or increase in IGF-I after 1 week. In the group as a whole, there was a downward trend in filtration fraction, but this was not significant. Plasma urea decreased significantly after 1 week, 18.2 (9.5–30) mmol/l on day 1 and 13.9 (5.3–22.5) mmol/l on day 8 ($P < 0.0002$), but returned to pre-treatment values by 3 months. Creatinine was unchanged after 1 week [274 (66–472) $\mu\text{mol/l}$ on day 1; 279 (69–486) $\mu\text{mol/l}$ on day 8], and there was no change in mean weight [22.2 (13.3–37.0) kg on day 1; 22.3 (13.4–36.8) kg on day 8] or blood pressure during this time (Table 2).

Results at 1 year

Fifteen children completed 1 year of treatment; the results are shown in Table 2 and Figs. 2–4. Mean IGF-I and GH remained elevated during treatment (Fig. 1). GFR did not change during the study, however there was a significant

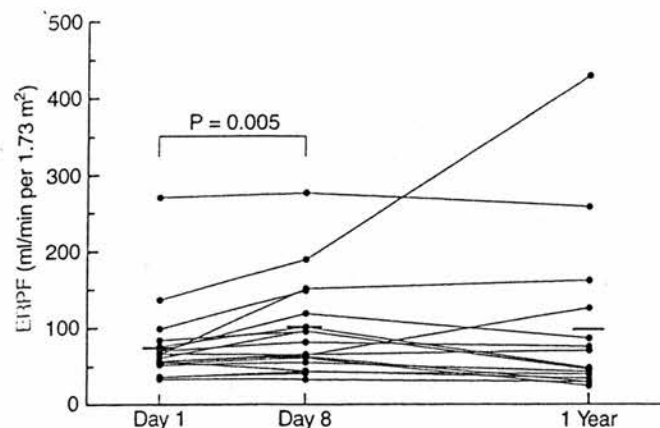


Fig. 3. Clearance of para-aminohippuric acid [effective renal plasma flow (ERPF)] in individual patients on day 1, day 8 and after 1 year of rhGH treatment. The mean value on each day is shown

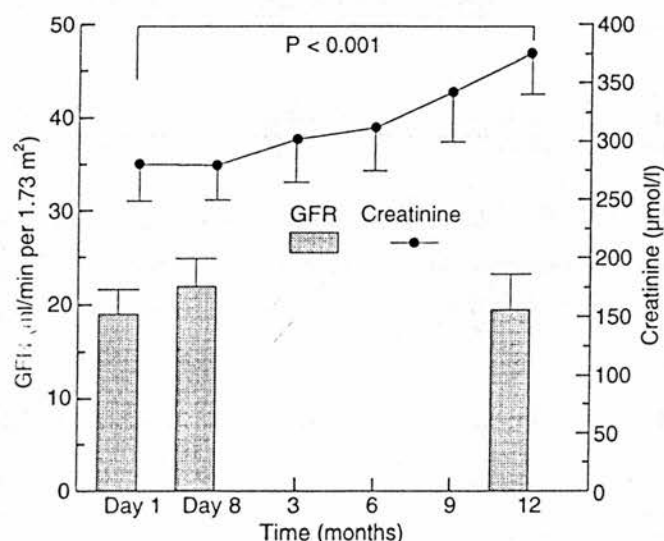


Fig. 4. Mean (SEM) GFR and mean creatinine during rhGH treatment

increase in creatinine ($P < 0.001$) (Fig. 4). Mean ERPF remained elevated after a year when compared with day 1, but this was not statistically significant. At 1 year ERPF was markedly elevated in 1 child, was higher than day 1 in 4, remained constant in 4, and declined by 20%–50% in 6. There was a downward trend in filtration fraction, but individually there was much variation; the decrease was not significant.

There was no correlation between baseline GFR, ERPF and the change in GFR and ERPF at 1 week or after 1 year. There was no correlation between absolute IGF-I (or IGF-I corrected for age and sex) and GFR or ERPF on day 1, day 8 or at 1 year, nor was there a relationship between the change in GFR or ERPF and the increase in IGF-I.

Mean systolic and diastolic blood pressures were unchanged by 1 year of rhGH (Table 2). Weight increased to 25.7 (15.5–39.2) kg after 1 year of treatment ($P < 0.0001$). Microalbuminuria, measured as an albumin (mg/l)/creatinine (mmol/l) ratio, was unchanged during the study mean \pm SD (0.08 ± 0.08 on day 1; 0.05 ± 0.05 on day 8; and 0.10 ± 0.12 at 1 year) in the 15 children with congenital

structural problems. The 3 children with FSGS had marked proteinuria.

Dietary intake of protein did not change during the study period: 2.1 (1.2–2.5) g/kg body weight protein on day 1; 1.9 (1.4–2.9) at 6 months; and 1.8 (1.4–2.1) at 1 year. Energy intake (MJ/kg body weight) was not affected by treatment: 0.36 (0.18–0.59) on day 1; 0.33 (0.27–0.47) at 6 months; and 0.36 (0.31–0.41) at 1 year.

Discussion

Many children with CRF grow poorly [20] despite elevated GH levels [21], which suggests that there is resistance to the actions of GH. Pharmacological doses of rhGH apparently overcome this and can effectively improve growth in these children [1–3]. However prolonged exposure to consistently high GH levels, such as are found in acromegaly and diabetes, results in an elevation of ERPF and GFR [22, 23]. This may be beneficial in CRF, but if hyperfiltration is a mechanism for progression of CRF in man [15], then rhGH treatment may in the longer term have a deleterious effect on renal function in CRF. However, patients with acromegaly do not go on to develop renal disease (in the absence of diabetes and hypertension) [24], whereas rodents exposed to prolonged elevation of GH levels develop glomerular sclerosis [25, 26].

In our patients there was no change in GFR during rhGH treatment, confirming a study in seven adults with CRF, none of whom showed any change in inulin clearance 24 h after 3 days of rhGH [10]. It also supports the findings of the rhGH trials in children with CRF [11–13], where GFR, as measured by single-injection inulin clearance, was unchanged after 6 months and 1 year [12], and where the change in calculated creatinine clearance after 24 months was no different on rhGH than placebo [13].

It is difficult to disentangle the long-term effects of rhGH treatment on renal function from the natural progression of CRF. Two children reached end-stage disease during treatment and required dialysis. It is not known if rhGH treatment affected their clinical course. Both children had FSGS and a rising creatinine before starting treatment. One child showed a 50% increase in ERPF after 1 week of rhGH; the other showed little change in ERPF. Four children reached end-stage disease in the year following the trial, so perhaps it was surprising that there was not a decline in mean GFR during the study.

Measurement of GFR by standard inulin clearance with urine collections in children can be difficult. It is not ethical to use bladder catheterisation, yet without it one cannot be sure that the child has completely emptied their bladder. Ensuring a high urine flow rate with the use of an oral water diuresis makes it easier to empty the bladder, and so reduces the risk of error. None of our children had bladder dysfunction. To verify the adequacy of bladder emptying, we also calculated baseline (pre GH) clearance of inulin by the constant infusion without urine collection method; at steady state the amount of inulin infused equals the amount cleared in the urine [27]. There was close correlation between these methods in our patients [28], confirming that bladder emptying was complete.

Plasma creatinine rose significantly despite a consistent GFR (Fig. 3), and presumably reflects an increase in muscle bulk. Fractional excretion of creatinine was unchanged by rhGH treatment (results not shown), therefore the increase in plasma creatinine was not due to a reduction in tubular secretion of creatinine. From our data it is clear that creatinine alone is an inadequate marker of GFR in children with CRF during rhGH treatment.

None of the previously mentioned studies [10–13] have looked at the effects of rhGH on ERPF in CRF in man. After 1 week of treatment we demonstrated an increase in mean ERPF, to a degree similar to that seen in individuals with normal renal function (approximately 25%) [7]. Three-day dietary assessments confirm that increased ERPF was not due to an increase in protein intake during the trial. Mean systolic and diastolic blood pressure did not change, therefore the increase in ERPF represents a decrease in renal vascular resistance.

In 4 children there was also an increase in GFR. It was not possible to predict the renal response to rhGH from baseline characteristics and in the group as a whole there was no relationship between the increase in circulating IGF-I and the changes in GFR and ERPF. Autocrine and paracrine actions of IGF-I within the kidney may be as, or even more, important than circulating IGF-I in mediating the renal effects of rhGH [29].

Our results are supported by a recent study of four adults with moderate CRF, all of whom showed an increase in GFR and ERPF in response to subcutaneous twice daily injections of rhIGF-I for 5 days [14]. GFR increased between 40%–70%, with a larger increase in ERPF, such that ultrafiltration fraction decreased in each of the four patients. The degree of renal insufficiency was less than in our patients. In two of these patients clearance of PAH remained elevated 10–12 days after stopping rhIGF-I, which the authors suggest may be due to glomerular hypertrophy, as circulating IGF-I had returned to normal. GFR had, however, returned to baseline. The renal response to rhGH and IGF-I in CRF in man appears to be variable.

Micropuncture work in normal rats infused with rIGF-I revealed an increase in GFR and ERPF due predominantly to an increase in efferent arteriolar dilatation and an increase in the ultrafiltration coefficient [30]. The relevance of these changes in CRF [31, 32] and their application to man are not clear, but it is interesting to speculate that changes within the glomerulus in CRF, dependent on the severity and aetiology of renal disease, may determine the response, if any, to rhGH or rhIGF-I. Alternatively, as a reduction in renal mass stimulates IGF-I production during compensatory hypertrophy in rodents [33, 34], there may already be increased IGF-I expression in the kidney in CRF which results in a different response to exogenous rGH or IGF-I. Renal hypertrophy per se may be important in the response to prolonged rGH exposure.

Whilst the evidence to date suggests that the renal effects of rGH are likely to be due to an effect of IGF-I on glomerular haemodynamics, it is not inconceivable that GH increases secretion of PAH by the proximal tubule. While this cannot be discounted from our data, neither can it be the whole explanation.

In small mammals, prolonged administration of GH results in glomerular hypertrophy and glomerular sclerosis, as has been shown in normal mice [25] and in renally impaired rats [32]. Interestingly prolonged exposure to IGF-I causes hypertrophy but not sclerosis of the glomerulus in transgenic mice [25, 35]. Micropuncture work has shown that glomerular capillary pressure is not raised in response to IGF-I in the normal rat [30], so perhaps sclerosis is due to an action of GH which is not mediated via IGF-I-induced haemodynamic changes [36]. As mentioned before, patients with acromegaly do not develop renal disease, despite exposure to high levels of GH. The renal response to GH in man would appear to be different from that in rodents; indeed the rodent reduced renal mass model may not be representative of CRF in man.

In conclusion, during a 1-year study of the use of rhGH in CRF, there was little change in mean GFR in those children completing treatment. Of the 18 children, 2 progressed to end-stage disease. After 1 week of treatment there was a significant increase in ERPF, and in some children ERPF remained elevated after 1 year. Whilst the mechanism and indeed the significance of the response is speculative at present, further study is needed to determine if rhGH affects the progression of CRF in children.

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Recombinant human growth hormone treatment in infants with chronic renal failure

H Maxwell, L Rees

Abstract

Poor growth is a particular problem for children with congenital renal disease. A one year trial of the use of recombinant human growth hormone (rhGH) in eight infants and young children with chronic renal failure is reported here. At entry bone age was less than 2 years, mean (range) chronological age 1.9 (1.3-2.7) years, and glomerular filtration rate (GFR) was 17 (9-42) ml/min/1.73 m². Height standard deviation score (SDS) was -3.3 (-4.6 to -2.0) and height velocity SDS was -1.3 (-3.1 to 0.7). One child was withdrawn when he received a renal transplant after 9.5 months. Two children required dialysis, but remained in the trial. Treatment with rhGH resulted in an increase in height SDS to -2.2 (-4.2 to -0.9), $p=0.0002$, and height velocity SDS to 1.1 (-0.7 to 2.6), $p=0.006$. There was no change in GFR and no serious adverse events. There was no effect on plasma lipids, calcium, phosphate, intact parathyroid hormone, or glucose. Alkaline phosphatase rose significantly. Thus rhGH improved growth in eight infants with chronic renal failure, with four children entering the normal range.

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Keywords: chronic renal failure, infants, growth, growth hormone.

Poor growth is a particular problem for children with congenital renal disease.¹ Growth in the first two years of life is greater than at any other time; by the age of 2 years children have attained half of their final adult height.² Ill health at this time results in a loss of height potential. While subsequent growth in the preschool and early school years is often normal in these children, catch up is rarely seen.³

Optimum conservative management, including provision of adequate energy, correction of electrolyte disturbance and acid-base abnormalities, and prevention of renal osteodystrophy goes some way towards improving growth, but many children remain below the normal range.^{1,3-7} Nasogastric or gastrostomy feeding to ensure adequate nutrition improves growth in some,^{3,8} but not all children.^{3,9}

Growth in the first two years of life is strongly influenced by nutrition. It is now appreciated that the influence of growth hormone is also important at this age; infants who are later diagnosed as having growth hormone

deficiency are shorter and lighter than normal.¹⁰

Recombinant human growth hormone (rhGH) improves growth in older children with chronic renal failure.^{11,12} We present here the results of a one year study of the use of rhGH in infants and young children with chronic renal failure, who were not growing despite good conservative management.

Methods

Criteria for entry to the study were: (1) chronic renal failure with a glomerular filtration rate (GFR) of less than 50 ml/min/1.73 m²; (2) a bone age of less than 2 years; (3) height less than -2 SD or a declining height standard deviation score (SDS) and no improvement in growth despite correction of fluid and electrolyte and acid-base balance, bone disease, and diet (including a trial of tube feeding when necessary); (4) absence of uncontrolled renal bone disease; (5) age less than 1 year at presentation of disease; (6) two previous height measurements in the last 6 months.

Children were excluded if there were other severe congenital abnormalities or if there had been a previous malignancy.

Growth hormone (Genotropin) (Pharmacia, Stockholm) was given as a daily subcutaneous injection (0.14 IU (0.05 mg)/kg/d, equivalent to 1 IU/kg/week) in the evening for one year.

Written parental consent was obtained.

PATIENTS

Eight children (three girls, five boys) were enrolled. Mean (range) chronological age was 1.9 (1.3-2.7) years. Six children had congenital structural problems, one had congenital nephrosis, and another had bilateral neonatal renal vein thrombosis. Calculated GFR¹³ at the start of treatment was 17 (9-42) ml/min/1.73 m².

Birth weight was 3.19 (2.24-4.50) kg at a mean gestational age of 36.7 (34-41) weeks. Five of the eight infants had a birth weight within the normal range; one child was small for dates and one of the two premature infants had a weight above that expected for gestational age. Growth data for the six months preceding the trial were recorded. At the time of entry to the trial, height SDS was -3.3 (-4.6 to -2.0). Height velocity SDS in the previous six months was -1.3 (-3.1 to 0.7).

MEASUREMENTS

The growth standards of Tanner *et al* were used to calculate the height and height velocity SDS.²

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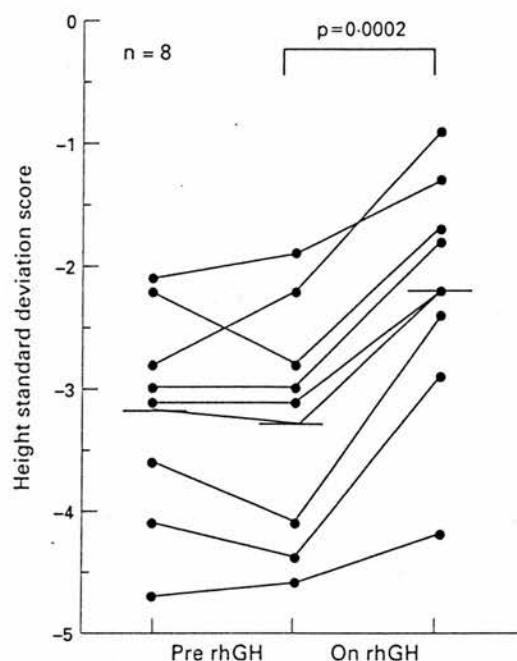


Figure 1 Height standard deviation score six months before, on day 1, and after one year of rhGH.

Children were seen three monthly, at which time blood was drawn for estimation of urea, creatinine and electrolytes, calcium, phosphate, intact parathyroid hormone (PTH), fasting glucose, insulin, cholesterol, and triglyceride. Height, weight, blood pressure, and adverse events were recorded.

Results are given as the mean (range). Statistical analysis was performed using a paired *t* test. Analysis of variance for repeated measures was used to compare multiple measurements in the same individuals.

Results

Seven of the eight children completed one year of treatment: one child was withdrawn when he received a renal transplant after 9.5 months of rhGH. Data from this child have been included in the analysis. Two children reached end stage renal failure and were begun on peritoneal dialysis, one after four months the other after 10 months. These children completed the study and their 1 year growth data have been included.

Height SDS increased from -3.3 (-4.6 to -2.0) to -2.2 (-4.2 to -0.9) ($p=0.0002$), fig 1; height velocity SDS increased from -1.3 (-2.7 to 0.7) to 1.1 (-0.7 to 2.6) ($p=0.006$), fig 2. Height SDS calculated six months before the study was -3.2 (-4.7 to -2.1), giving a mean increase in height SDS during treatment of 1.1 (0.4 to 1.7) compared to 0.08 (-0.6 to 0.6) in the preceding six months ($p=0.0016$).

Response to rhGH in this group of patients was unrelated to GFR. The greatest increment in height SDS was seen in the youngest children ($r=-0.794$, $p=0.019$) (fig 3).

There was no change in blood pressure (table 1). Weight increased from 9.5 (7.5 – 13.8) kg to 10.6 (8.3 – 12.9) at six months and 11.7 (8.9 – 13.8) after one year ($p=0.008$ v day1). Weight, expressed as per cent of the ideal weight for height, was 98% (79–118) at the

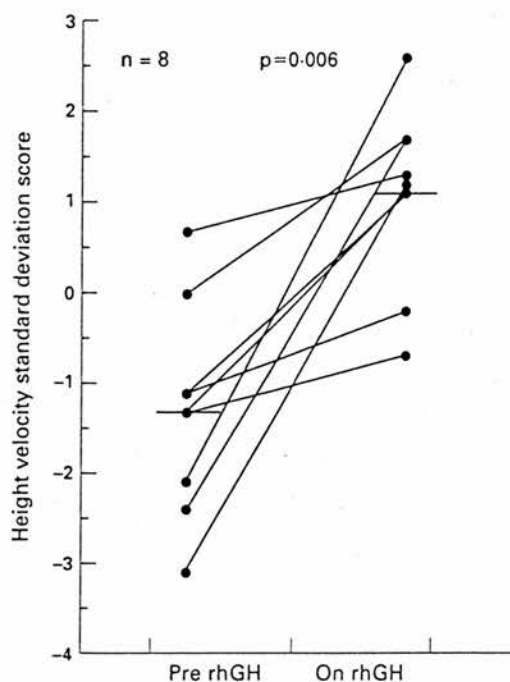


Figure 2 Height velocity standard deviation score before and during rhGH treatment.

start of the study, and 96% (79–127) after one year.

Calculated GFR (ml/min/1.73 m^2) in the children remaining on conservative management was unchanged by rhGH treatment, at 17 (9–42) on day 1 and 17 (6–34) after treatment. Results for creatinine, urea, and blood pressure are shown in table 1. There were no significant changes in any of these variables. There were no consistent changes in plasma calcium, phosphate, intact PTH, triglyceride, or cholesterol (table 2). Alkaline phosphatase was raised after six months ($p=0.05$) and remained raised at one year (table 2). Plasma glucose remained constant, but there was a trend for an increase in fasting insulin (table 2). This was not statistically significant, presumably because the sample number was small.

No serious adverse events occurred during the trial.

Discussion

In renal disease poor growth in the first two years of life can result in a significant loss of height potential.¹⁹ Growth thereafter in the preschool and early school years is often normal, so that the children grow parallel to,

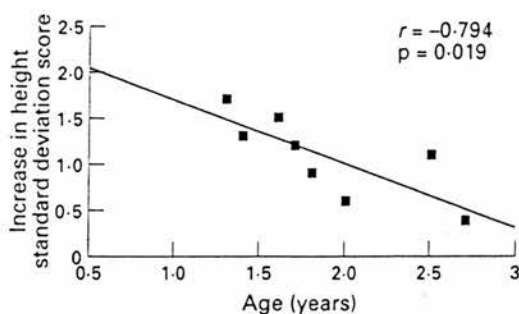


Figure 3 Increment in height standard deviation score during treatment plotted against age at start of treatment.

Table 1 Values for urea, creatinine, and blood pressure (BP) before and after recombinant human growth hormone (rhGH) treatment (mean and range)

	Day 1	6 Months	After rhGH
Creatinine (mmol/l)	213 (70-320)	259 (95-488)	276 (100-555)
Urea (mmol/l)	13.3 (4-19.2)	11.6 (1.7-20.4)	12.6 (1.4-21.9)
Systolic BP	99 (84-116)	103 (85-126)	93 (80-110)
Diastolic BP	65 (51-85)	61 (50-71)	54 (46-60)

but well below the third centile.^{3,4} Catch up growth at this time is rare.³

At entry to the trial, mean height SDS was -3.3 despite a mean age of just under 2 years. A prospective study of growth in infancy in chronic renal failure reported that the major loss in height and weight SDS occurred within the first six months⁹; this loss can be as great as 0.6 SD per month.⁵ Delay in diagnosis can therefore result in a significant loss of growth potential. Optimum conservative management, with correction of electrolytes, acid-base status, and calcium metabolism and provision of adequate nutrition improves growth in some^{3,8} but not all children with chronic renal failure.^{9,12} Improvement is more common in children who present to a paediatric nephrologist below the age of 2 years than those who present at an older age.³

Traditionally, it has been thought that growth in infancy is influenced primarily by nutrition, but recently growth hormone has been recognised as a contributing factor at this age.¹⁰ The use of energy supplements in infants with chronic renal failure, by nasogastric or gastrostomy feeding if necessary, does not consistently improve growth.^{3,8,9} In a placebo controlled trial, rhGH has been shown to improve growth in older prepubertal children with chronic renal failure,¹⁴ and to maintain an improved growth velocity for up to 3 to 5 years, but there is little published data of its use in infants and young children.¹⁵ The results of rhGH treatment in two boys aged less than 2 years with chronic renal failure was reported in abstract form (Linne *et al*, *Pediatr Nephrol* 1992; 6: C118). Both showed a good response to treatment.

Height SDS did not change in the six months before the trial, while it increased from -3.3 to -2.2 during rhGH treatment. Each child showed an improvement, with four children entering the normal range. The increment in height SDS represents good catch up growth, and interestingly the greatest improvement was seen in the youngest children (fig 3).

The dramatic changes in height velocity in the first two years of life make interpretation of growth data difficult within this age group.

Height velocity SDS has been used to allow comparison between height velocities at different ages. Ideally comparison should be with a placebo control group, but ethically this is difficult. With the small number of children involved in this study, it was decided to treat all children. Height SDS was used as the criterion for entry to the trial. Annualisation to compare height velocity at this age can be misleading because of the changing velocity, yet observing growth for one year to obtain an accurate velocity measurement will only lead to a delay in treatment.

Despite the low height SDS before treatment, mean height velocity SDS was within the normal range. This suggests that reduction in height SDS had indeed started early. Furthermore remarkable catch up growth on rhGH was seen despite relatively normal height velocity SDS values during rhGH treatment (most values were within the normal range, albeit in the upper half rather than the lower). Although this is partly an auxiological phenomenon resulting from the high rates of growth seen in infancy, it appears to be easier to achieve catch up growth at this time compared with later childhood,^{11,12,15} a situation which is also reported following renal transplantation.^{16,17}

Growth after infancy, in the late preschool and early school years, is often normal in chronic renal failure^{3,6}; it remains to be determined whether or not children treated in infancy with rhGH can maintain their position within the normal range without further rhGH treatment. Perhaps a short course of treatment in infancy can achieve as much benefit as a longer course later in childhood. The benefits have to be weighed against the potential trauma of daily injections in this age group. However these children may need to remain on rhGH to maintain a height within the normal range.

There was no change in calculated GFR in the children who remained on conservative management. Patients with acromegaly have large kidneys that hyperfilter,¹³ and rhGH increases GFR and effective renal plasma flow (ERPF) when given to subjects with normal kidneys.¹⁹ We have previously shown that one year of rhGH treatment has no effect on GFR, although there is an increase in ERPF after one week, which is no longer evident at one year.²⁰ The long term implications of these findings are unclear. Two of the children were started on dialysis; it is not known whether rhGH treatment was implicated. The group included several children with severely reduced renal function; one child was withdrawn from the study when he received a renal transplant.

Table 2 Plasma biochemical variables before and after recombinant human growth hormone (rhGH) treatment (mean and range)

	Normal range	Day 1	6 Months	After rhGH
Calcium (mmol/l)	(2.12-2.65)	2.54 (2.31-2.74)	2.62 (2.38-2.79)	2.54 (2.20-2.94)
Phosphate (mmol/l)	(1.1-1.8)	1.47 (1.10-1.96)	1.51 (0.9-2.10)	1.64 (1.30-2.20)
PTH (ng/l)	(15-65)	140 (35-436)	96 (22-568)	75 (2-570)
Alkaline phosphatase (U/l)	(150-900)	432 (178-870)	636 (265-1589)*	610 (270-1118)*
Fasting glucose (mmol/l)	(3.0-6.0)	4.8 (3.9-5.6)	4.8 (3.5-5.8)	5.0 (4.0-6.3)
Fasting insulin (IU/l)		23.1 (4.3-68)	34.9 (4.0-75)	47.4 (17.3-92)
Cholesterol (mmol/l)	(1.9-7.0)	5.3 (3.3-8.7)	5.2 (3.9-6.4)	5 (3.4-8.2)
Triglyceride (mmol/l)	(0.3-1.8)	2.7 (1.7-4.3)	3.4 (2.1-6.5)	1.4 (0.8-2.0)

*p=0.05 v day 1.

It has been much debated whether growth is related to GFR *per se*. It has been reported by one group¹ that growth declines when the GFR falls below 25 ml/min/1.73 m², and by others that good growth can be achieved in some children even at a very low GFR values.^{5,6} We were unable to find a relation between growth rate and GFR either before or after rhGH treatment, although it is noted that our group of patients is small. The two children who were started on peritoneal dialysis had the smallest increase in height SDS.

No adverse events were reported during the trial. There was no significant change in serum calcium, phosphate, or PTH, but there was an increase in alkaline phosphatase during rhGH treatment, as had been reported previously during accelerated growth in older children on rhGH.¹¹ Baseline values of cholesterol and triglycerides were variable, with no consistent changes during treatment. There was a trend towards an increase in fasting insulin during rhGH, but no change in fasting glucose. The same findings have been reported previously with the use of rhGH in chronic renal failure; fasting insulin returned to baseline during the second year of treatment.¹⁴

In summary, one year of rhGH resulted in a marked improvement in height SDS in a group of infants and young children with chronic renal failure. Two children reached end stage disease and required dialysis. GFR in the remaining children was unaffected by rhGH treatment. There were no serious adverse events. Further study is necessary to determine the role and optimum timing of rhGH treatment in renal disease.

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Effects of recombinant human growth hormone on renal function in children with renal transplants

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Objective: To provide accurate measurement of renal function during treatment with recombinant human growth hormone (rhGH).

Methods: We measured glomerular filtration rate and effective renal plasma flow by clearance of inulin and para-aminohippuric acid before rhGH therapy, after 1 week, and then at 6-month intervals for up to 2 years of treatment in 16 children (mean (SD) age = 13.1 (2.2) years; glomerular filtration rate = 52 (27) ml/min per 1.73 m²). The mean (SD) time from transplantation was 6.5 (3.6) years.

Results: Linear growth velocity during rhGH therapy increased from 4.0 (1.8) to 8.8 (2.6) cm/yr ($p < 0.0001$). One child was withdrawn after 9 months because of abnormal glucose tolerance, and another child received a second renal transplant after 18 months. Glomerular filtration rate increased to 57 (29) ml/min per 1.73 m² at 1 week ($p = 0.004$), remained improved at 6 months (63 (30); $p = 0.013$), but was not significantly better at 1 year (59 (33)). Effective renal plasma flow on day 1 was 237 (127) ml/min per 1.73 m² and was unchanged on day 8 (244 (123)), at 6 months (271 (149)), and after 1 year (269 (157)). During the study there was no significant change in filtration fraction, blood pressure, or kidney volume, and excretion of microalbumin and *N*-acetylglucosaminidase was unaltered. There was one rejection episode per 14.8 patient-months in the year before treatment, 1 per 18.9 patient-months during the first year of treatment, and 1 per 13 patient-months during the second year of rhGH therapy.

Conclusion: Treatment with rhGH improves growth in children with renal transplants. Glomerular filtration rate was increased after 1 week and 6 months of rhGH therapy but returned to baseline values thereafter. The data indicate the need for long-term follow-up of children with renal transplants who are receiving rhGH. (J PEDIATR 1996;128:177-83)

Recombinant human growth hormone has been shown to improve growth in children with renal transplants, and its use

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is now becoming more widespread. However, there are concerns regarding the effect of rhGH on graft function. Patients with acromegaly have large kidneys that hyperfilter.¹ When given to adults with normal renal function, rhGH increases glomerular filtration rate and effective renal plasma flow.² Growth hormone may also affect the immune system,³ thereby increasing the incidence of transplant rejection.⁴

Published reports give conflicting results of the effect of rhGH on graft function.⁵⁻¹³ It is important to ensure that renal function is not compromised at the expense of improved

ERPF	Effective renal plasma flow
GFR	Glomerular filtration rate
GH	Growth hormone
IGF-I	Insulin-like growth factor I
PAH	Para-aminohippuric acid
rhGH	Recombinant human growth hormone

growth, not least because graft function itself has been shown to have an effect on growth.¹⁴ We have studied the effects of rhGH on GFR, ERPF, kidney volume, blood pressure, and the excretion of microalbumin and *N*-acetylglucosaminidase in 16 children with renal transplants, after 1 week and then at 6-month intervals for up to 2 years of treatment. Seven children acted as their own control subjects in the year before treatment.

METHODS

Sixteen children (four girls) from two pediatric nephrology centers in London were entered into the study, which lasted for 2 years and was part of a large international multicenter trial that is still in progress. More detailed renal function studies were performed in this subgroup of patients, who are therefore described separately. In the first year, children were randomly selected either to receive treatment or to have no treatment; in the second year, all children received rhGH. Results for all 16 children during the year of rhGH therapy are reported and are compared with data from the previous year. Patient details are given in Table I.

All children had either a height less than the 3rd percentile and a linear growth velocity less than the 75th percentile or a height less than the 25th percentile and a linear growth velocity less than the 25th percentile during the preceding year. They had received their grafts more than 1 year previously and had had stable graft function for the 6 months before the trial. The initial diagnosis was congenital structural problems in 9 children and congenital nephrotic syndrome in 3, and the remaining 4 children had cystinosis, atypical hemolytic-uremic syndrome, nephronophthisis, and undetermined glomerulopathy, respectively. Two grafts were from living related donors; 12 were first grafts, 3 were second grafts, and 1 was a third transplant.

Nine children received triple immunosuppression consisting of azathioprine, 60 mg/m² per day, cyclosporine (at a dose sufficient to maintain plasma levels between 50 and 100 ng/ml), and prednisolone; seven children received only azathioprine and prednisolone. Prednisolone was given at a mean dose of 9.8 (range, 5.2 to 20.1) mg/m² on alternate days to 15 children; one child received 7.6 mg/m² daily. Recombinant human GH (Genotropin, from Pharmacia) was given at a dose of 0.14 IU/kg per day (equivalent to 1 IU/kg per week or 0.35 mg/kg per week) as a subcutaneous injection every evening.

Retrospective growth data and creatinine values were available for all children for the year preceding entry into the trial. Approval was granted by ethics committees at both centers. Written informed consent was obtained from the parents after verbal consent was given by the children.

Children were seen on day 1 (before treatment), day 8 (after 1 week of rhGH therapy), every 3 months for up to 1 year, and then every 6 months thereafter. On each occasion, and while the children were in the fasting state, blood was sampled for estimation of urea, creatinine, GH, and insulin-like growth factor I values. With the exception of day 1, these samples were drawn approximately 12 hours after the preceding dose of rhGH. Height was measured with a wall-mounted stadiometer by the same observer. Blood pressure was measured in the sitting position.

On days 1 and 8 and then at 6-month intervals, GFR and ERPF were estimated by clearance of inulin (Inutest 25%, Laevosan-Gesellschaft GmbH, Linz/Donau, Germany) and para-aminohippuric acid (aminohippurate sodium, 20%; Merck, Sharp & Dohme, West Point, Pa.), respectively, with the use of a standard clearance with urine collection technique, as described previously.¹⁵ Briefly, a dose of inulin and PAH was given, followed by an infusion of both; the concentration of this solution was calculated so as to achieve constant plasma levels. After 1 hour, four 40-minute urine collections were obtained. The GFR and ERPF values were calculated from the average of the four collections.

Kidney volume was measured yearly by one of us (A.J.S.S.), using either an ATL Mark IV (Advanced Technology Laboratories, Inc., Bothell, Wash.) or an Acuson 128 (Acuson, Inc., Mountain View, Calif.) ultrasound machine, with 3.5 or 5 MHz sector probes. When the whole length of the kidney could not be included in the image, a stand-off gel block was used. Repeated measurements of renal length were made until a maximum was reached. Multiple depth and width measurements were taken and averaged. Kidney volume (measured in milliliters) was estimated by means of the formula for an ellipse.¹⁶

Rejection episodes were diagnosed by the clinician in charge, and were assumed to have occurred when there was an increase in the baseline creatinine value of more than 10% on two occasions, in the absence of a high cyclosporine level (120 ng/ml; therapeutic range, 50 to 100 ng/ml), dehydration, infection, or obstruction. Treatment consisted of a 3-day course of orally administered prednisolone (3 mg/kg per day). A renal biopsy was performed if the creatinine value did not return to baseline after this treatment.

Inulin was measured enzymatically,¹⁷ PAH was determined by the Bratton Marshall reaction,¹⁸ creatinine by an enzymatic method using an RA1000 automatic analyzer (Technicon Instruments Co., Ltd., Basingstoke, United Kingdom), and IGF-I by radioimmunoassay after acid-eth-

anol extraction. This last method of extraction does not completely remove IGF binding proteins,¹⁹ which can then interfere in the assay. The extent of interference appears to depend on the particular antibody being used. In our assay, acid chromatography, when compared with acid-ethanol extraction, gave comparable results in chronic renal failure ($r = 0.979$; $p < 0.0001$), and acid-ethanol gave slightly lower results ($y = 0.906x - 0.049$). Growth hormone was measured by a two-site immunoradiometric assay.

Ten children had urine samples analyzed for microalbumin by a two-site ("sandwich") enzyme-linked immunosorbent assay.²⁰ Polyclonal rabbit anti-human albumin immunoglobulin was used both as the capture antibody and, when conjugated to horseradish peroxidase, as signal antibody (Dako Corp., High Wycombe, United Kingdom). Intraassay and interassay coefficients of variation were 4.2% and 11.9% at 10 mg/L, respectively. The reference range of 0 to 10 mg/mmol creatinine was obtained from healthy children.²⁰

N-Acetylglucosaminidase was measured by an automated spectrophotometric method with *p*-nitrophenyl- β -D-glucosaminide as substrate (Sigma, Poole, Dorset, United Kingdom).²¹ Intraassay and interassay coefficients of variation were 2.5% and 5.8%, respectively, at 3.9 μ mol *p*-nitrophenol per hour per millimole of creatinine. The reference range of 0 to 100 μ mol *p*-nitrophenol per hour per millimole of creatinine was obtained from healthy children.²⁰

Three-day dietary assessments were made at 6-month intervals.

Control data. Seven children were randomly selected to receive no treatment in the first year. Their results during this year will be summarized briefly.

Statistical analysis was performed with a paired Student *t* test; significance was confirmed with a Wilcoxon paired rank sum test. Comparison of different groups was performed with an unpaired *t* test. Two-way analysis of variance was used to compare repeated measurements in the same group of patients.

RESULTS

Fifteen children completed 1 year, seven completed 18 months, and six have completed 2 years of rhGH treatment. Fasting hyperglycemia developed in patient 6, in association with a raised concentration of hemoglobin A_{1c} after 9 months, at which time rhGH treatment was stopped.

Two children (patients 7 and 11) had poor graft function at the start of the trial; both had biopsy-proven chronic rejection. Patient 11 received a second graft after 18 months of treatment with rhGH; patient 7 completed 2 years of rhGH therapy but required hemodialysis 6 months after completion of the trial. Patient 13 had pyelonephritis after 18 months of

Table I. Patient details (N = 16)

	Value (range)
Age (yr)	13.1 (9.4-16.4)
GFR (ml/min/1.73 m ²)	52 (18-117)
Time from transplantation (yr)	6.5 (2.0-11.8)
HtSDS on day 1	-2.9 (-4.5 to -1.6)
HtSDS at 1 yr	-2.4 (-4.3 to -0.9)*
GV (cm/yr) before rhGH therapy	4.0 (0.8-8.1)
GV (cm/yr) during rhGH therapy	8.3 (3-12.5)†

HtSDS, Height standard deviation score; GV, [linear] growth velocity.

* $p < 0.001$ versus day 1.

† $p < 0.0001$ versus before rhGH therapy.

rhGH and had a permanent deterioration of graft function during this episode. Patient 16 had hypertension and hyponatremia after oral water diuresis during the inulin clearance study on day 1; subsequent measurement of renal function was by single-injection inulin clearance only.

Growth. Linear growth velocity doubled in the group receiving rhGH, and was associated with a significant improvement in the height standard deviation score (Table I). Growth velocity during treatment was positively correlated with GFR at the start of treatment ($r = 0.637$; $p = 0.006$). Five patients were pubertal at the start of rhGH therapy; Tanner stage was 2 to 3 at the start of rhGH therapy and 2 to 4 after 1 year. The other 11 children were prepubertal at the start of the trial; 1 child progressed into puberty during treatment.

Glomerular filtration rate. The GFR increased from 52 (range, 18 to 117) to 57 (20 to 118) ml/min per 1.73 m² after 1 week of rhGH therapy ($p = 0.004$). The GFR remained improved at 6 months to 63 (18 to 133) ml/min per 1.73 m² ($p = 0.013$) but not at 1 year (59 [15 to 113]) (Fig. 1). By analysis of variance, there was a significant increase in GFR with time ($p = 0.036$). There was no further significant change in GFR in those children treated for 2 years: 53 (17 to 65) ml/min per 1.73 m² at 18 months ($n = 7$), and 38 (11 to 51) at 2 years ($n = 5$). Despite an increase in GFR, there was an increase in plasma creatinine concentration from 110 (range, 43 to 221) μ mol/L to 139 (64 to 317) after 1 year of treatment ($p = 0.028$) (Table II), presumably because of an increase in muscle bulk. The blood urea concentration decreased after 1 week of rhGH therapy but returned to pre-treatment values by 3 months (Table II).

Effective renal plasma flow. There was no change in ERPF, as measured by clearance of PAH, between day 1 (237 [82 to 495] ml/min per 1.73 m²) and day 8 (244 [88 to 539]). There was no significant change after 6 months (271 [95 to 572]; $p = 0.065$) or after 1 year (269 [56 to 538]). There was no significant change in ERPF in those children who received rhGH for 2 years: 285 (54 to 598) ml/min per 1.73 m² at 18 months ($n = 7$), and 219 (41 to 469) at 2 years ($n = 5$). There was no change in filtration fraction: 0.23 (0.14

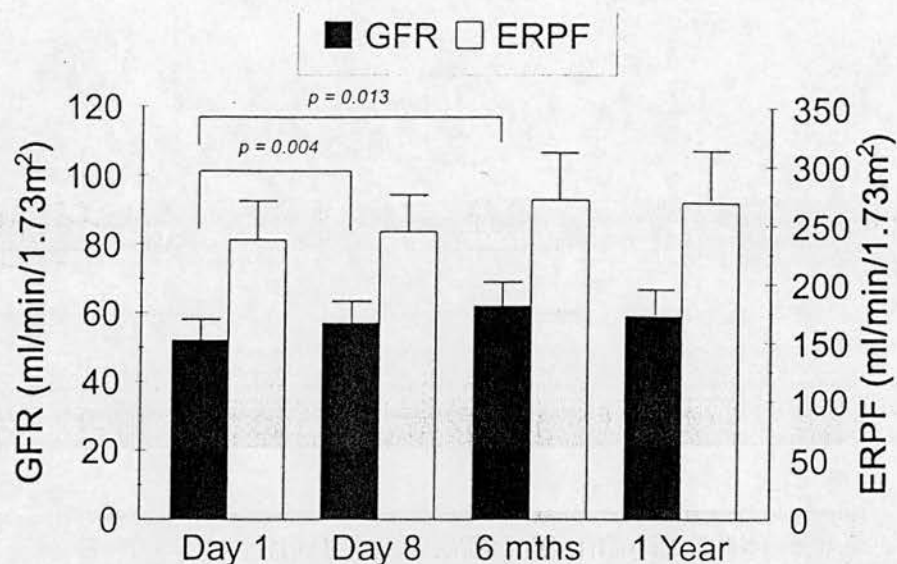


Figure. GFR and ERPF during the year of rhGH treatment. Results are shown as mean and SE.

Table II. Results during rhGH treatment

	Day 1	Day 8	At 3 mo	At 6 mo	At 9 mo	At 1 yr
Creatinine ($\mu\text{mol/L}$)	110 (43-221)	111 (37-248)	114 (33-235)	114 (37-240)	126 (47-252)	133 (47-317)*
Urea (mmol/L)	12.1 (6.0-26.7)	10 (3.0-22.0)†	10.8 (2.7-22.5)	11.9 (3.0-33.1)	11.6 (3.2-22.2)	12.4 (5.3-29.5)
GH (mU/L)‡	6.2 (1.1-14)	23.0 (5.0-52.1)†	13.1 (1.5-30.6)	17.6 (3.7-43.6)	12.8 (1.0-43.6)	20.5 (0.7-59.3)
IGF-I (ng/mL)§	242 (95-410)	540 (117-1002)†	613 (166-903)	590 (105-891)	649 (89-958)	681 (424-948)
Blood pressure (mm Hg)						
Systolic	110 (100-124)	114 (100-128)	111 (95-130)	114 (105-125)	108 (90-125)	115 (100-130)
Diastolic	70 (50-80)	76 (50-95)	71 (51-100)	75 (60-90)	68 (60-80)	69 (60-80)

Values are mean (range).

* $p = 0.03$ versus day 1.

† $p < 0.001$ versus day 1.

‡Fasting GH: normal range is less than 10 mU/L.

§IGF-I: normal range is age related.

to 0.42) on day 1, 0.25 (0.14 to 0.48) at 1 week, 0.25 (0.13 to 0.36) at 6 months, and 0.24 (0.15 to 0.32) at 1 year.

Kidney volumes. Kidney volume was measured in 10 patients before and during rhGH treatment. Mean volume was 128 (range, 57 to 221) ml on day 1 and 133 (57 to 227) after 1 year ($p = 0.14$). The change in kidney volume was no different from that seen in five children studied during the year of no treatment: 114 (76 to 148) ml on day 1 and 123 (99 to 150) after 1 year ($p = 0.38$).

Blood pressure. There was no significant change in systolic or diastolic blood pressure during rhGH treatment (Table II).

Transplant dysfunction. There was no increase in the incidence of transplant dysfunction during rhGH treatment; there was 1 episode per 14.8 patient-months in the year before rhGH therapy; 1/18.9 patient-months during the first year of rhGH therapy, and 1/13 patient-months during the

second year of rhGH therapy (Table III). In the first treatment year there were 10 episodes of increase in serum creatinine concentration, which were assumed to be related to rejection and were treated accordingly. Six episodes occurred during the second year.

Urinary protein excretion. Three patients had elevated microalbumin excretion before starting rhGH treatment. There was no significant change in albumin excretion during therapy: on day 1 the mean (SE) value was 17.3 (11.6) mg/mmol creatinine; at 6 months, 13.8 (6.7); and at 1 year, 13.1 (6.1). There was also no change in the pattern of urinary *N*-acetylglucosaminidase excretion: on day 1 it was 106 (27) μmol *p*-nitrophenol per hour per millimole creatinine; at 6 months, 120 (20); and at 1 year, 123 (26). On day 1, four patients had elevated levels that persisted with treatment.

Dietary assessments. There was no significant change in mean (SE) dietary protein intake: 1.3 (0.2) gm protein N per

kilogram of body weight on day 1, 1.1 (0.3) at 6 months, and 1.3 (0.1) at 1 year. Energy intake was likewise unchanged: 37.9 (5.3) kcal/kg on day 1, 31.6 (5.8) after 6 months, and 41.5 (7.7) at 1 year.

Growth hormone and insulin-like growth factor I. Serum GH and IGF-I levels increased after 1 week of rhGH therapy and remained elevated thereafter (Table II). There was no relationship between baseline IGF-I or increase in IGF-I and the changes in renal function in individual patients.

Control data. Values for GFR, ERPF, creatinine, and urea did not change in the seven children studied during their year of no treatment (data not shown). Blood pressure, protein excretion, and kidney volume were also unchanged. Linear growth velocity was 4.5 (2.8 to 7.8) cm/yr during the year before the trial, 4.1 (1.4 to 5.6) during the year of no treatment, and 8.5 (5.3 to 11.1) during the first year of rhGH therapy ($p < 0.004$ vs year of no treatment).

DISCUSSION

We found that rhGH treatment in children with renal transplants resulted in an increase in GFR after 1 week and 6 months, with a return to pretreatment values by 1 year. There was no significant change in ERPF or in kidney volume. There are a number of mechanisms whereby rhGH could affect renal function. First, GH, through the action of IGF-I, increases GFR and ERPF in the normal kidney; micropuncture studies in the rat demonstrate that this is due to an effect on glomerular hemodynamics.²² Second, altered renal function could be related to hypertrophy of the kidney; prolonged exposure to GH causes renal enlargement, as seen in acromegaly²³ and in transgenic mice secreting high levels of GH.²⁴ Hypopituitary mice undergoing unilateral nephrectomy have reduced hypertrophy of the remaining kidney.²⁵ Third, transgenic mice with prolonged exposure to GH (but not to IGF-I) have glomerulosclerosis and renal failure.²⁴ Finally, rhGH may have an effect on the immune system, and in renal transplantation could precipitate acute rejection or cause progression of chronic rejection.³

It is difficult to differentiate among these mechanisms. We looked at the effects of rhGH after 1 week to see whether there is a rise in GFR and ERPF, such as that seen in normal adults. This is the case for GFR but not for ERPF. The increase in GFR was still evident at 6 months. In health it has been proposed that this may be due to an alteration in glomerular hemodynamics, as demonstrated by micropuncture studies in rats.²² This may also be the explanation in our children, because it is unlikely that any of the other mechanisms outlined above would be acting at 1 week. At 6 months, increased GFR could still be due to altered hemodynamics, or possibly also to hypertrophy. With time, however, other influences, such as progression of chronic rejection,

Table III. Episodes of transplant dysfunction

Patient No.	Year before rhGH	First year of rhGH	Second year of rhGH
1	0	0	0
2	0	0	0
3	0	0	
4	1	0	0
5	0	0	
6	0	0	
7*	0	0	1
8	0	0	
9	5	1	2
10	2	1	
11*	1	3	2
12	0	0	
13	0	0	1
14	2	3	
15	0	0	
16	2	2†	

*Biopsy-proven chronic rejection before rhGH therapy.

†Chronic rejection on biopsy during rhGH therapy.

have to be taken into account. Despite the increase in GFR, there was also an increase in the serum creatinine concentration, presumably reflecting increased muscle bulk. We previously showed a similar phenomenon in children with chronic renal failure receiving rhGH.¹⁵

Few studies have formally measured GFR during rhGH therapy. One reported no significant change in GFR, as measured by single-dose inulin clearance,⁵ and the other¹³ reported no significant changes in GFR as measured by inulin clearance. Other studies of rhGH therapy after renal transplantation quote changes in creatinine clearance or changes in the slope of an inverse creatinine (1/creatinine) plot; however, changes in the creatinine value do not always reflect a change in GFR. Calculated GFR²⁶ takes some account of this; a multicenter European study¹⁰ showed no significant change in calculated GFR after 1 year. Another study⁷ showed no significant change in creatinine concentration in 19 children after 1 year, another reported a decrease in creatinine clearance in 5 of 9 patients,¹² and yet another reported a decrease in calculated GFR in 3 but an increase in the other 2 patients.⁹ Interpretation of these studies is hampered both by the lack of formal measurement of GFR and by the heterogeneity within patient groups. Slowly growing children tend to have poorer renal function to start with (in one study a rise in the creatinine value of 90 $\mu\text{mol/L}$ was associated with a decrease in the height standard deviation score of -0.17^{14}). Moreover, GFR decreases with the number of rejection episodes and with the time since transplantation,²⁷ so changes in GFR during studies such as these may be related to factors other than rhGH.

It is more difficult to determine the effects of rhGH on the

incidence of rejection episodes. Transplant dysfunction that appears clinically to be rejection is treated in our centers with a 3-day course of high oral doses of prednisolone; we proceed to a biopsy only if there is no response to this treatment. This does not allow assessment of the true incidence of rejection, but a similar policy was used in both centers before and during the trial. The incidence of transplant dysfunction was not different during rhGH treatment in comparison with the year before. From the literature, the use of rhGH has been associated with biopsy-proven acute rejection in children with previously stable graft function at variable times after commencing rhGH therapy,^{8, 10, 28, 29} and also with the progression of chronic rejection.^{7, 12} In one report, two children with previously diagnosed chronic rejection had an increase in the serum creatinine concentration after the use of rhGH. The creatinine level decreased when rhGH therapy was stopped, which suggests a functional effect rather than irreversible changes.³⁰

Treatment with rhGH could also have an effect on tubular function, as well as glomerular function; there are receptors for GH on proximal tubular cells.³¹ To investigate this effect, we measured urinary excretion of *N*-acetylglucosaminidase, a renal tubular enzyme. Excretion was unchanged during rhGH treatment, as was the excretion of albumin.

We conclude that there was an increase in GFR after 1 week and after 6 months of rhGH therapy in children with renal transplants; GFR returned to baseline by 1 year. We found no change in ERPF, kidney volume, or rate of clinical rejection episodes, and no change in urinary protein excretion. Therapy with rhGH had an acute effect on graft function, which has not been demonstrated previously; whether this is important in terms of long-term graft function remains to be determined. The results of large multicenter trials, in progress at the moment, may provide the answer.

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RECOMBINANT HUMAN GROWTH HORMONE (rhGH) TREATMENT OF INFANTS AND YOUNG CHILDREN WITH CHRONIC RENAL FAILURE

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SUMMARY Infants with chronic renal failure often grow badly despite medical management. rhGH treatment results in good catch-up growth and should be used in infants who do not respond to conservative management.

Growth can be a significant problem in renal disease, particularly for children with congenital renal problems.¹ Growth is fastest in the first two years of life, and ill-health at this time will result in the child's height rapidly falling away from the centiles.² Nasogastric or gastrostomy feeding improves growth in some, but not all, of these children,³ and this has led to interest in the use of rhGH in this age group.⁴

There were nine infants with chronic renal failure in the UK trial of recombinant human growth hormone (rhGH) in renal disease, and data from these children were analysed separately.⁵

PATIENT DETAILS

Criteria for entry to the study included a GFR of $<50\text{ml/min/1.73m}^2$, a bone age of less than two years and a height less than the 3rd centile or a declining height SDS, despite correction of electrolyte and acid-base balance, bone disease and the provision of adequate calories (by nasogastric or gastrostomy feeding where necessary). All children had developed renal failure before the age of one year. rhGH (1IU/kg/week) was given as daily subcutaneous injection in the evening for one year.

Mean (SD) age was 2.0 (0.5) years, range 1.3–2.7 years, and mean height SDS was -3.3 (0.9). As height velocity changes dramatically during the first two years of life, change in height velocity SDS (HVSDS) was used in preference to height velocity. HVSDS in the year before the trial was -1.3 (1.2). Calculated GFR was 17 (12) ml/min/1.73m^2 at the start of treatment.

RESULTS

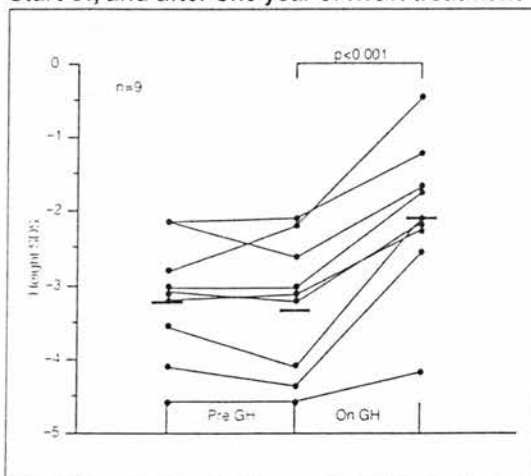
Seven children completed a full year of rhGH treatment. Two children received renal transplants during the study, and two others required peritoneal dialysis. Annualised data from all nine children have been included in the analysis.

HVSDS increased to 1.4 (1.1) during treatment ($p=0.004$), and height SDS to -2.1 (1.0) ($p<0.001$) (Figure 1). GFR at one year was unchanged at 16 (11). The best response to treatment was seen in the youngest children ($r = -0.864$; $p=0.002$) (Figure 2).

DISCUSSION

HVSDS increased in all nine patients and was associated with a marked improvement in height

Figure 1. Height SDS six months before, at the start of, and after one year of rhGH treatment



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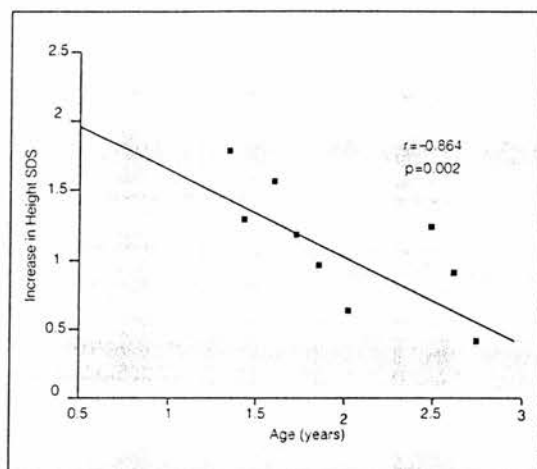


Figure 2. Increase in height SDS, plotted against age at start of treatment

SDS (1.2SD), with four children entering the normal range. Fine et al reported similar findings in a two-year study of rhGH in infants with CRF; mean height SDS increased from -3.0 to -1.1.⁴

Growth is fastest during infancy and early childhood, and while nutrition improves growth in some children, this is not universal. Mean height SDS at

the start of treatment was -3.3, despite a mean age of only 1.9 years and despite optimum conservative management, showing that children with congenital renal disease can rapidly fall away from the centiles at this time.

Although growth rate after early childhood is often normal in chronic renal failure patients, catch-up growth is rare. It will be interesting to see if infants treated with rhGH can maintain their height SDS or if they require continuing rhGH treatment. Whatever, rhGH treatment at this time results in good catch-up growth and it should be used in those infants who do not respond to conservative management.

ACKNOWLEDGEMENT

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USE OF RECOMBINANT HUMAN GROWTH HORMONE (rhGH) IN PUBERTAL PATIENTS WITH RENAL DISEASE

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SUMMARY Growth during puberty in renal failure and following renal transplantation is often poor. Treatment with rhGH improved height SDS in 13 pubertal children with renal disease.

Recombinant human growth hormone (rhGH) is now being used for short stature due to renal disease. Efficacy in prepubertal children has been widely reported,^{1,4} but efficacy in pubertal children is less clear. Growth during puberty is often poor in children with renal disease.⁵ In the combined UK trial of rhGH in renal disease, 13 children were in early puberty at the start of the study; we present their data here.

METHODS

Children were classified as chronic renal failure (CRF), dialysis, or post-renal transplantation. The CRF group had a glomerular filtration rate (GFR) of $<50\text{ml/min/1.73m}^2$ (for at least one year), and the children in the dialysis group had all been on either haemodialysis or peritoneal dialysis for more than six months. The transplant group had stable graft function and a minimum GFR of 20ml/min/1.73m^2 .

Early puberty was defined in boys as a testicular volume $\geq 4\text{ml}$ and $\leq 10\text{ml}$, and in girls as Tanner breast stage 2 or 3.

Mean patient details (range) are given in Table 1. The transplant group received prednisolone $8.2 (0-15.2)\text{mg/m}^2$ on alternate days. The children were treated with rhGH at a dose of 1IU/kg/week , given as

a daily subcutaneous injection in the evening. Renal function, rejection episodes and adverse events were monitored.

RESULTS

Height velocity (Figure 1) and height SDS (Figure 2) increased significantly during treatment. There was no change in calculated GFR in the CRF group ($19 (10-30)\text{ml/min/1.73m}^2$ on day one; $18 (11-26)$ at one year), or in inulin clearance in the transplant group ($48 (32-78)\text{ml/min/1.73m}^2$ on day one; $48 (27-84)$ after one year).

There were five rejection episodes in three of the seven transplant children during the study. In the year before the trial, there were four such episodes in two (of the same three) children.

DISCUSSION

Height velocity increased in 11 of the 13 children during rhGH therapy, corresponding to an increase in height SDS of 0.5, $p=0.004$. In this group of children, rhGH had no effect on renal function or rejection rate.

While these results are encouraging, it is difficult to differentiate the effects of rhGH from those of the endogenous pubertal growth spurt in pubertal

Table 1. Patient details

	Age (years)	HV (cm/yr)	HtSDS
CRF (n=3)	13.8 (13.0-14.4)	4.7 (3.4-5.8)	-3.1 (-4.2 to -2.3)
Dialysis (n=3)	14.5 (12.4-15.7)	4.6 (0.3-8.4)	-2.8 (-4.8 to -1.0)
Transplant (n=7)	15.2 (12.4-19.8)	4.2 (1.3-8.1)	-2.5 (-4.5 to -1.6)

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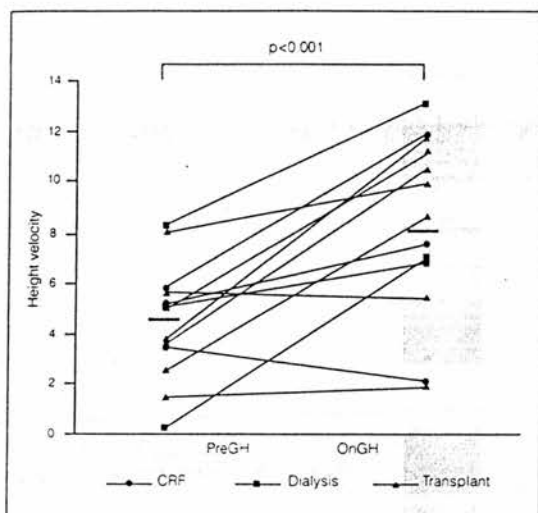


Figure 1. Height velocity during the year before and the year of rhGH treatment

patients. A controlled trial is needed, but unfortunately, in any one study, the numbers are too small. The transplant children are part of a multicentre controlled study which should provide two-year growth data. It is only when these children reach final adult height, however, that the efficacy of rhGH during puberty can be definitively determined.

ACKNOWLEDGEMENT

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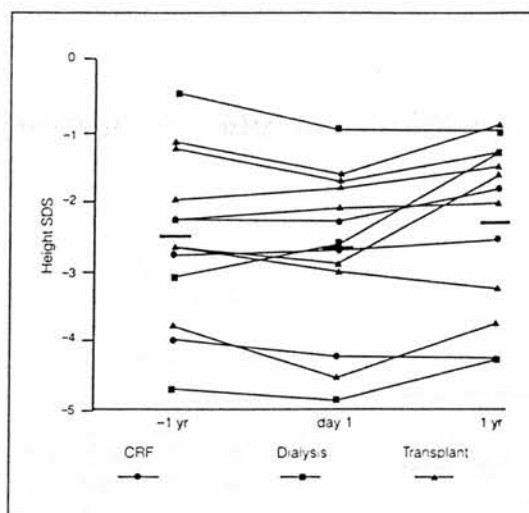


Figure 2. Height SDS one year before the trial, on day one and after one year of rhGH treatment

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Review article

Factors influencing the response to growth hormone in children with renal disease

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Abstract. The effects of age, height velocity over the preceding year, glomerular filtration rate (GFR) and prednisolone dose on growth response have been assessed by single and multiple linear regression analysis in 23 prepubertal children [age, mean (SD), 8.2 (2.5) years] with chronic renal failure (CRF) and 16 prepubertal children [12.1 (2.3) years] with renal transplants treated for 1 year with recombinant human growth hormone (rhGH), 30 U/m² per week. Height velocity [mean (SD), cm/year] increased from 4.7 (1.3) to 9.7 (2.1) ($P < 0.0001$) in the CRF group and 3.1 (1.6) to 7.3 (2.8) ($P < 0.0001$) in the transplant group. In the CRF group, there was a correlation between age and height velocity, both in the pretreatment year ($r = -0.755$, $P < 0.0001$) and during treatment ($r = -0.421$, $P = 0.045$). There was no correlation between pretreatment height velocity or GFR and response to rhGH. In the transplanted children height velocity during the treatment year correlated with age ($r = -0.647$, $P = 0.007$), prednisolone dose ($r = -0.689$, $P = 0.003$), GFR ($r = 0.542$, $P = 0.030$) and pretreatment height velocity ($r = 0.655$, $P = 0.006$). Multiple regression analysis showed prednisolone dose and age to be the most important predictors of response.

Key words: Recombinant human growth hormone – Chronic renal failure – Transplant – Growth – Prednisolone

Introduction

Over the last few years there has been considerable development in the understanding of the endocrinology of growth failure in renal disease. The identification of growth

Table 1. Clinical details of the children treated with recombinant human growth hormone (rhGH)^a

	Age (years)	HtSDS	GFR	Height velocity (cm/year)	
				pre rhGH	during rhGH
CRF (n = 23)	8.2 (2.5)	-2.9 (0.6)	20 (12)	4.7 (1.3)	9.7 (2.1)*
Transplanted (n = 16)	12.1 (2.3)	-3.2 (0.9)	57 (30)	3.1 (1.6)	7.3 (1.8)*

HtSDS, Height standard deviation score; GFR, glomerular filtration rate; CRF, chronic renal failure

* $P < 0.0001$ compared with pretreatment year

^a Mean (SD)

hormone (GH) resistance in chronic renal failure (CRF) led to the rationale for trials of recombinant human GH (rhGH), which have proved to be successful, at least in the short term. However, in every series there are children who do not grow as well as would be predicted. We have analysed the factors influencing the growth response to rhGH in children with CRF and with renal transplants.

Patients and methods

We have treated with rhGH 16 prepubertal (Tanner stage 1) children (12 boys) with renal transplants and 23 children (17 boys) with conservatively managed CRF, as part of two studies of the use of rhGH in renal disease [1–3]. Only 4 children on dialysis have been treated, therefore they have not been included in the analysis. Pubertal children and those entering puberty (i.e. progressing beyond stage 1 for genital, breast or pubic hair) during treatment have also been excluded, as interpretation of growth during this time is confounded by the pubertal growth spurt. All the children were over 3 years of age, with height standard deviation scores (SDS) of more than 2 SD below the mean, or height velocity SDS more than 1 SD below the mean. All had attended the clinic for at least 18 months so that metabolic control had been optimised and there were reliable height data for comparison of growth velocity over the year before treatment with the year of rhGH treatment. None had diabetes, uncontrolled bone disease or abnormal liver or thyroid function tests. The clinical details of the children are shown

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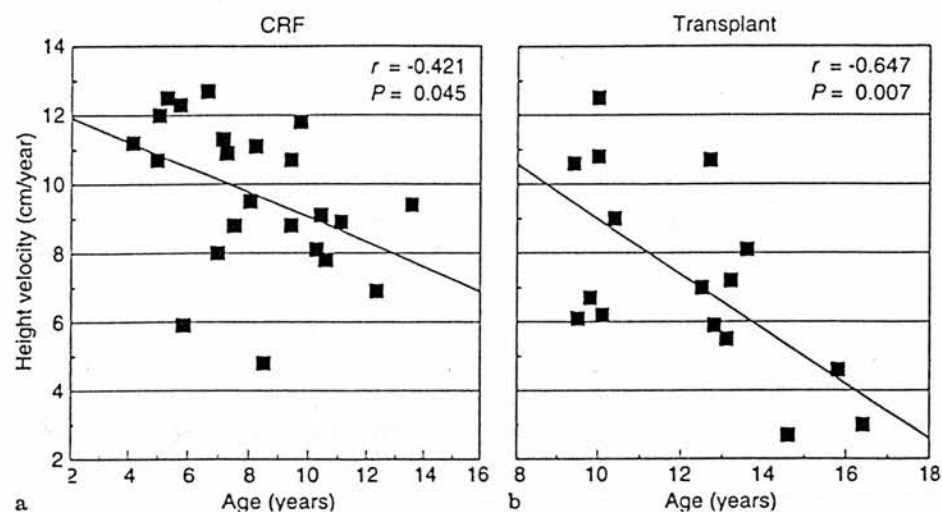


Fig. 1. Effect of age at the start of 1 year of treatment with recombinant human growth hormone (rhGH), 30 U/m² per week, on height velocity during the treatment year in: a 23 prepubertal children with chronic renal failure (CRF) and b 17 prepubertal transplanted children

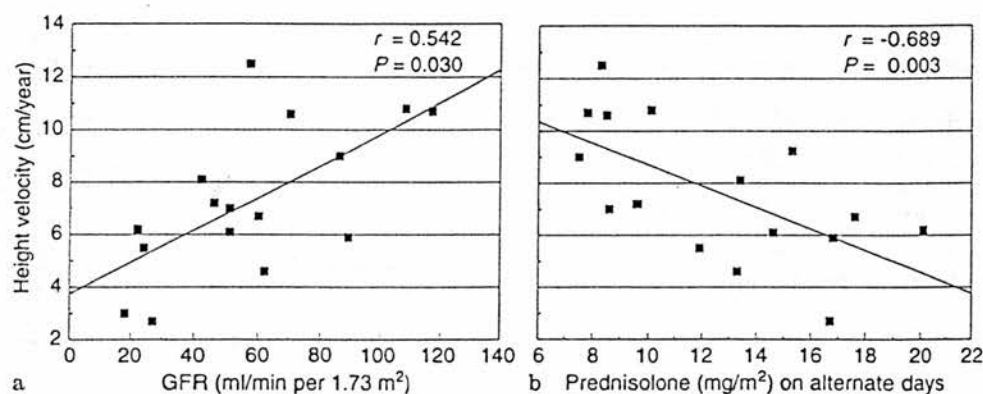


Fig. 2. Effect on height velocity during 1 year of treatment with rhGH (30 U/m² per week) in 17 prepubertal transplanted children of: a the glomerular filtration rate (GFR) at the start of the year and b the mean steroid dose over the year

in Table 1. The mean (SD) prednisolone dose used for immunosuppression was 12.5 (4.0) mg/m² on alternate days, given as a single dose in the morning. In the first 12 children studied, glomerular filtration rate (GFR) was calculated using the Schwartz formula [4]; in the following 27 it was measured by inulin clearance. rhGH (Pharmacia) was given at a dose of 30 U/m² per week in daily doses subcutaneously. Results over the 1st year of treatment have been analysed. Student's *t*-tests and single and multiple linear regression were used for analyses. $P < 0.05$ was accepted as significant.

Results

Rate of growth increased significantly over the year of rhGH treatment compared with the previous year in children with CRF ($P < 0.0001$) and post transplant ($P < 0.0001$) (Table 1).

CRF patients

Over the year before rhGH, there was a correlation of height velocity with both age ($r = -0.755$, $P < 0.0001$) and GFR ($r = 0.467$, $P = 0.025$). During rhGH therapy, height velocity correlated with age ($r = -0.421$, $P = 0.045$) (Fig. 1a) but not GFR. There was no correlation between age and GFR, or between height velocity before and during rhGH treatment.

Transplanted children

Over the year before rhGH, there was no correlation between height velocity and either age or GFR, but there was with prednisolone dose ($r = -0.603$, $P = 0.013$). During the year of rhGH, there was a correlation between height velocity and age ($r = -0.647$, $P = 0.007$, Fig. 1b), GFR ($r = 0.542$, $P = 0.030$, Fig. 2a), prednisolone dose ($r = -0.689$, $P = 0.003$, Fig. 2b) and height velocity during the year before rhGH ($r = 0.655$, $P = 0.006$). There was no correlation between GFR and prednisolone dose. Using multiple regression, only prednisolone dose and age had a significant effect on growth response; GFR and pretreatment growth velocity no longer had a significant influence.

Discussion

Factors influencing the response to rhGH have been studied in children treated with rhGH for many differing reasons. In all cases, there seems to be an age-dependent effect, with younger children responding the best [5]. A positive correlation between age and response to rhGH has also been demonstrated in renal disease [6]. This relationship extends from infancy [7] to puberty [6], and has been confirmed in this study in both CRF and after transplantation.

Another important factor influencing the response to rhGH is the pretreatment growth velocity. In short normal children, growth rate rather than absolute size is related to endogenous GH secretion, and those with the lowest height velocity and therefore the lowest endogenous GH secretion have the best response to rhGH [5]. We were unable to find such a relationship in our patients; indeed in the transplanted children, the slower growing children had the poorer response to rhGH, although when the results were standardised for age, there was no correlation between the height velocity SDS over the pretreatment year and the increment in height velocity SDS. Whereas Wühl et al. [6] found a negative correlation between prestudy height velocity SDS and increment in height velocity SDS, this correlation was positive in the study by Hokken-Koelega et al. [8]. The reasons for the different findings are unclear, but may be due to the small number of patients studied. If there is a positive correlation between height velocity at a given age and response to rhGH, this may reflect the different mechanism of growth failure in renal disease, rather than GH deficiency, there is GH resistance which is presumably the most severe in the most slowly growing.

Children with renal disease have many factors that might be expected to affect their response to rhGH, such as the severity of their renal failure, their nutritional state, accompanying metabolic abnormalities and, in transplanted children, the dose of steroids. Although a correlation between GFR and response to rhGH has not been demonstrated in CRF in both this and other studies [6] (and varying control of these metabolic factors may be one reason for this), children on dialysis do less well than those on conservative management [9]. A correlation between growth response and GFR was seen, however, in our patients after transplantation, although this correlation was no longer significant when the effects of steroid dose and age were taken into account. The most important factor to affect growth response in the transplanted children appears to be the dose of prednisolone, even in children on an alternate-day regimen.

The growth-suppressing effects of steroid therapy are well recognised. As part of the cause is a depressant effect on GH secretion, there are theoretical reasons why transplanted children might respond better than children with CRF, who have normal or high GH levels [10]. However, although most studies have shown an improvement in growth, this is not usually to the same extent as children with conservatively managed CRF [1, 7]. Our patients, like those in the study of Wühl et al. [6], responded equally well to rhGH whether they were transplanted or in CRF.

In conclusion, children with conservatively managed CRF respond better to rhGH the younger they are. After

transplantation, young age, good renal function, higher pretreatment growth velocity and low-steroid dose are associated with a better response to rhGH. However, age and dose of prednisolone are the most significant predictors of response. Therapeutic options for improving growth in the older transplanted child on prednisolone therapy, with a low GFR and a low pretreatment growth velocity, are currently limited.

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The hypothalamo-pituitary-growth hormone insulin-like growth factor 1 axis in children with chronic renal failure

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The hypothalamo-pituitary-growth hormone-IGF1 axis in children with chronic renal failure. Growth hormone (GH) levels are high in children with chronic renal failure (CRF), and there is increased hypothalamic sensitivity to the factors that normally modulate GH secretion. GH binding protein (GHBP) is decreased, which may reflect decreased GH receptor activity. IGFBP3 levels are normal in both CRF and transplanted children when measured by immunoradiometric assay, but intact IGFBP3 is low in transplanted children with an excess of small fragments, measured by Western immunoblotting. These fragments may interfere with IGF1 bioactivity.

Normal growth depends on the complex interaction of genetic influences, adequate nutrition, intact endocrine control, and tissue responsiveness. Over the last few years there have been considerable developments in our understanding of the hypothalamo-pituitary-growth hormone (GH)-insulin-like growth factor (IGF) axis, both in health and disease. This paper will review these developments.

The hypothalamo-pituitary-GH-IGF axis in health

The hypothalamus produces GH releasing hormone (GHRH) and GH release inhibiting hormone (GHRH) which together modulate the secretion of GH so that it is released in pulses from the pituitary (Fig. 1). The pulsatility of GH release seems to be necessary for normal growth.

Approximately half of the GH circulates bound to a specific high affinity binding protein, growth hormone binding protein (GHBP). GHBP contains amino acid sequences that are identical to the extracellular GH-binding domain of the GH receptor, and it is thought that GHBP arises by proteolytic cleavage from the GH receptor. The avidity and specificity of GHBP is similar to that of the GH receptor itself. It is thought, therefore, that GHBP may reflect GH receptor activity [1].

As well as modulating the interaction of GH with tissue receptors, GHBP holds the pulse of GH in the circulation and in the extracellular space and decreases its clearance [1].

The circulating GH-GHBP complex acts in two ways: firstly by a direct effect on the chondrocyte, resulting in local production of IGF1, which through a paracrine mechanism causes the clonal expansion of differentiated chondrocytes, thus leading to bone growth; and secondly it stimulates the liver to produce IGF1 and its insulin-like growth factor binding protein (IGFBP). It is

believed to be free, rather than bound, IGF1 that is able to interact with chondrocytes. The relative importance of the endocrine, paracrine and autocrine activities of IGF1 remains speculative.

IGFBP3 is the major IGFBP in serum. The IGFBPs extend the half-life of the IGF peptides, transport them to target cells and modulate their interaction with surface membrane receptors. IGFBP3 reflects GH secretion and sensitivity [2].

IGFBPs inhibit IGF action, both in tissue culture and *in vivo*. This is thought to be due to competition with IGF receptors for IGF peptides. IGF1 binds to the IGFBP3 with higher affinity than with its receptor. Recently specific receptors for IGFBPs on the cell membranes have been demonstrated, suggesting a potential mechanism for direct IGFBP action [2].

There are proteases that degrade IGFBP3 into fragments which have decreased affinity for IGF1, but the physiological significance of protease activity is not clear.

Disorders of growth may, therefore, result from abnormalities at the hypothalamus, the anterior pituitary, the GH receptor, and the interaction of IGFs with chondrocytes in epiphyseal growth cartilage.

The hypothalamic control of GHRH and GHRH in CRF

Original studies of GH in CRF showed fasting GH levels to be high, with an exaggerated and prolonged response to hypoglycemia, arginine and GHRH [3]. Administration of somatostatin causes a rapid decrease of plasma GH [4]. These findings suggest that in CRF there is an increased hypothalamic sensitivity and responsiveness to the usual stimuli that induce GH secretion.

The pituitary

GH peaks are normal in frequency in CRF but increased in amplitude with high basal levels. The worsening of CRF is associated with a rise in GH baseline levels, and flattening of peaks, presumably due to decreased renal clearance and prolonged half life of GH (Fig. 2) [5].

The GH receptor

We have measured GHBP in 13 children with CRF. All the children had been attending clinic for at least a year so that there was reliable height data for that period, and metabolic control had been optimized. The mean calculated GFR was 17 with a range of 6 to 36 ml/min/1.73 m².

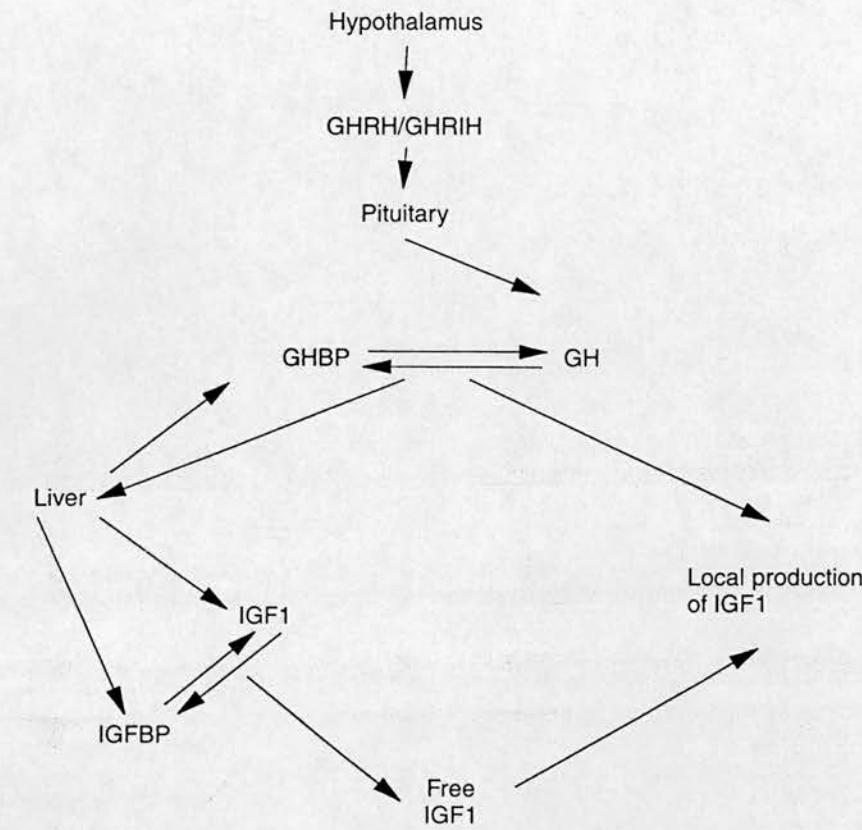


Fig. 1. Current theory for growth in health.

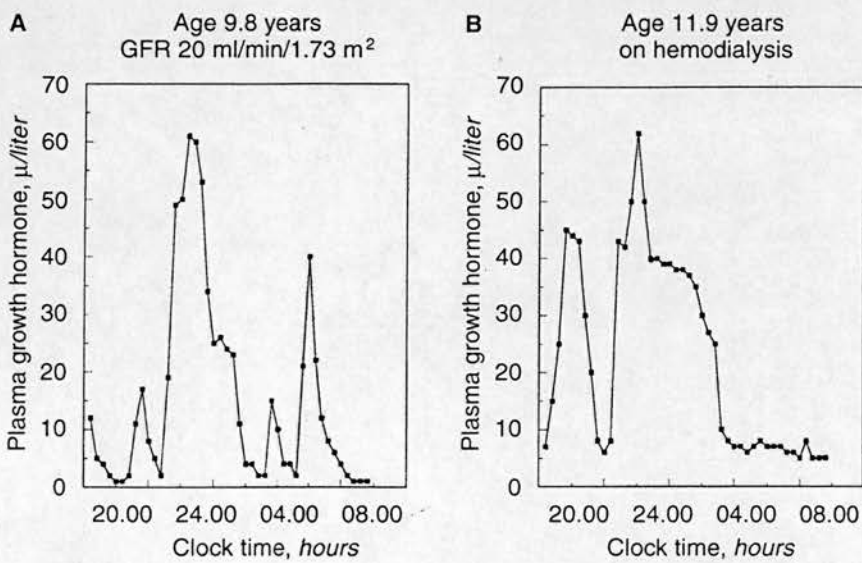


Fig. 2. Overnight GH secretion in two boys in CRF.

They were matched for age and height SDS with a group of 11 short normal children who were attending the growth clinic (Table 1).

A sample of blood was collected in the morning after an overnight fast. GH and IGF1 were measured by radioimmunoassay. GHBP was measured by incubation of serum with hGH labeled with ¹²⁵I. The sample was then analyzed by gel filtration. This results in two peaks, representing the free hGH and the

Table 1. Measurement of GHBP in 13 children with a GFR (mean, range) of 17 (6–36) ml/min/1.73 m², and 11 short normal children

	CRF	Short normal
Age years	8.2 (6.1 to 12.0)	8.6 (5.3 to 10.5)
Ht SDS	−2.0 (−5.0 to 1.6)	−2.3 (−3.3 to −1.1)
cm/year	5.9 (4.0 to 9.2)	4.7 (3.9 to 5.4)
Ht Vel SDS	0.1 (−2.4 to 2.5)	−0.8 (−1.9 to 0.0)

Data are mean (range).

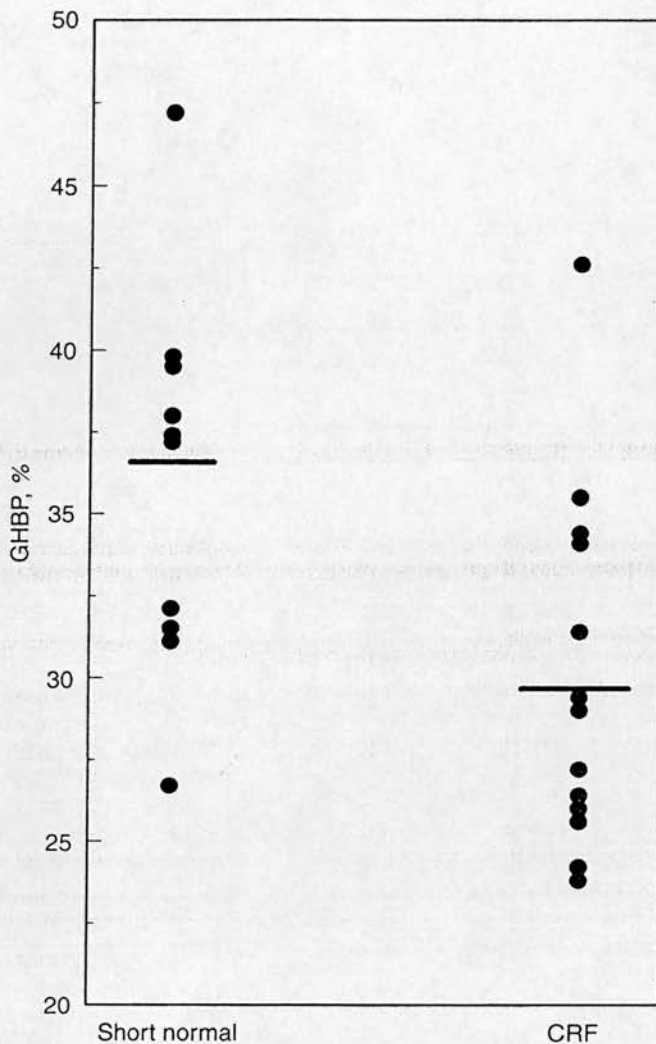


Fig. 3. GHBP levels in 13 children with CRF and 11 short normal children. $P = 0.03$.

bound GH. Binding is expressed as percentage of the total radioactivity in the higher molecular weight peak [7]. Plasma hemoglobin, urea, bicarbonate, parathyroid hormone (PTH) were also measured as markers of metabolic control.

Figure 3 shows the mean and distribution of GHBP in the two groups of children. GHBP levels were significantly lower in the children with CRF than the short normal children ($P = 0.03$). There was, however, no difference between the two groups for GH or IGF1 (Table 2).

No correlations were found between GHBP and growth, or with GFR or metabolic control.

GHBP has also been shown to be low in children on dialysis [6].

Low GHBP in CRF may represent decreased GH receptor expression, and may reflect GH resistance in CRF. Decreased GH receptor expression may be due to circulating toxins, or to down-regulation of the GH receptor due to increased circulating GH levels in CRF.

Table 2. Results of hormone analyses in the children with CRF and the short normal children

	CDR	Short normal children	P
GH μliter	9.2 (2.2–28.3)	9.6 (1.1–33.5)	NS
IGF1 μml	0.7 (0.1–1.3)	0.6 (0.2–0.8)	NS
GHBP %	29.5 (23.8–42.6)	36.4 (26.7–47.2)	0.03

Data are mean (range).

Table 3. Details of the patients treated with rhGH for one year

	CRF	Transplanted
Age years	8.7 (4.1–13.9)	13.1 (9.4–16.4)
GFR ml/min/1.73 m^2	20 (9–58)	52 (18–117)
Change in HtSDS during rhGH	0.8 (0–1.4)	0.5 (–0.2 to 1.3)

Data are mean (range).

Table 4. IGFBP3 during rhGH treatment

Group	Day 1	6 Months	1 Year
CRF			
IRMA mg/liter	3.8 (0.7)	6.0 (1.3) ^a	6.5 (1.8) ^a
WLB AU/mm^2	102 (74)	139 (79) ^a	182 (93) ^a
Transplant			
IRMA mg/liter	4.0 (1.0)	5.4 (1.5) ^a	5.5 (1.3) ^a
WLB AU/mm^2	56 (45)	113 (103) ^a	168 (86) ^a

Results are shown as mean and SD.

^a $P < 0.001$ vs. day 1

The interaction of IGF1 with chondrocytes

The predominating current theory for poor growth in renal disease is that IGFBP3, which is renally excreted, is inadequately cleared in CRF. This leads to high circulating levels of IGFBP3 and its fragments, which are also thought to bind IGF1, resulting in less free IGF1 and therefore poor growth [8]. However, not all investigators have been able to find high IGFBP3 levels in CRF [9].

Most investigators who have looked at IGF1 bioactivity, measured by incorporation of sulphate into cartilage cells in culture, have found it to be reduced in CRF and in patients on steroid therapy [8, 10, 11]. IGF1 measured by radioimmunoassay is normal in both CRF and renal transplantation [12]. Whatever the mechanism for poor growth in CRF, rhGH increases height velocity [12].

We have looked in detail at IGFBP3 and its circulating fragments, at the start and over the course of a year of rhGH therapy, and also at IGF1 measured by radioimmunoassay (Maxwell et al, submitted for publication).

There were 16 children with transplants and 17 with CRF. The transplant group had received their grafts a mean (range) of 5.9 (1.9 to 11.8) years previously. The prednisolone dose was 8.8 (5.2 to 9.6) mg/m^2 on alternate days. The rhGH dose was 1 $\mu\text{kg/week}$. The transplant group children were slightly older and had better renal function (Table 3).

Before treatment, IGFBP3 was normal when measured by immunoradiometric assay (IRMA) [13]. However, when assessed by Western ligand blotting (WLB) [14], which measures intact

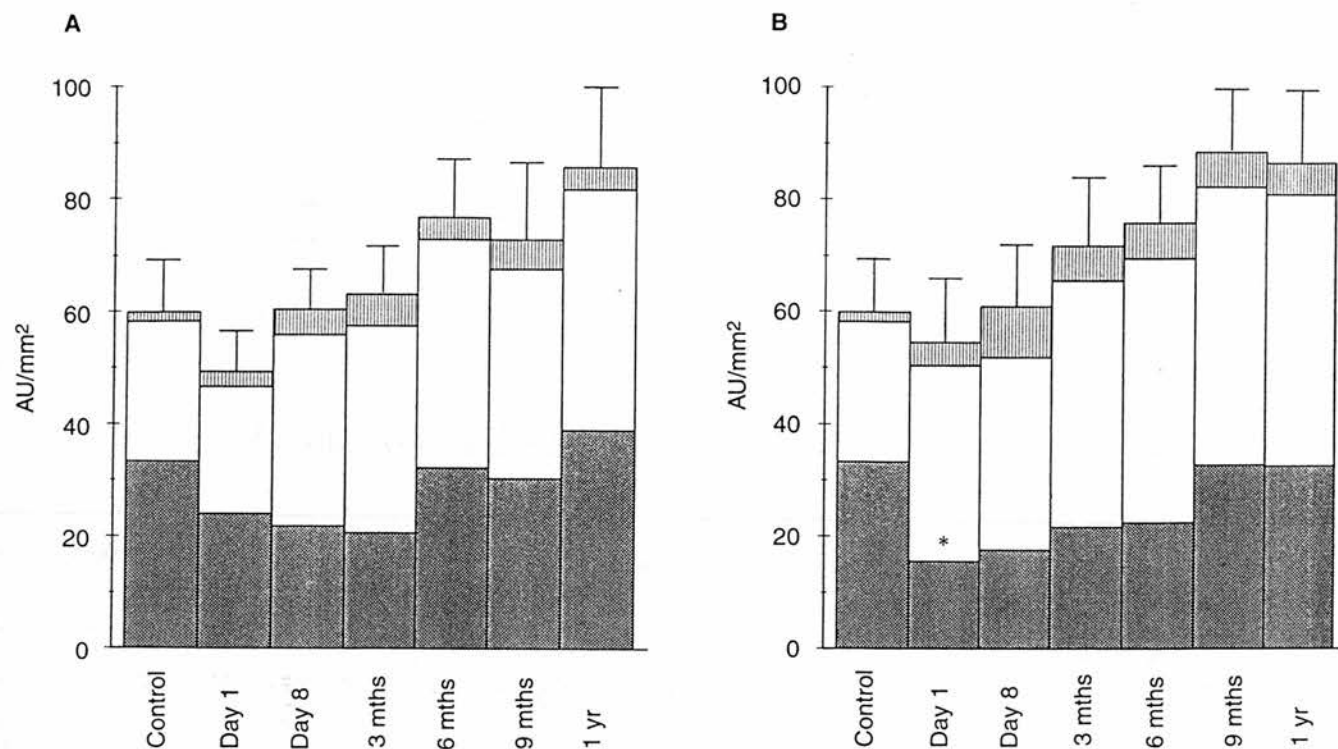


Fig. 4. Measurement of IGFBP3 and its fragments using Western Immunoblotting in 6 children with CRF (A) and 6 children with renal transplants (B) in comparison to controls, and over a year of rhGH therapy. Symbols are: (■) intact; (□) 30 kD; (▨) fragments. * $P < 0.03$ vs. controls.

IGFBP3, the levels in the transplant patients were significantly lower than in CRF. IGFBP3, as measured by both assays, rose over the year of rhGH treatment, so that IGFBP3 by WLB was similar in CRF and post-transplant (Table 4). A previous study using WLB in 7 adolescents on hemodialysis has also found levels of intact IGFBP3 to be similar to controls, but there was an increase of smaller fragments [13].

Sera from 6 children with CRF and 6 with transplants were arbitrarily selected to be analyzed by Western immunoblotting [15], which detects intact IGFBP3, the 30 kD fragment and small fragments of IGFBP3. Again there was no difference between controls and the CRF patients, but there was an increase in fragments in the transplant patients in comparison to controls. The levels of IGFBP3 increased over the year of rhGH therapy, and the proportion of fragments decreased so that they did not differ from the controls (Fig. 4).

For many years it has been recognized that IGF1 bioactivity is decreased in patients on steroid therapy, and it is interesting to speculate that these IGFBP3 fragments may be responsible for the decreased bioactivity, either by interfering directly with the IGF1 receptor, or by binding IGF1.

As steroids are catabolic, we looked to see whether the presence of circulating fragments could be due to increased protease activity [16]. However, protease activity in both the CRF and the transplant group was no different from controls.

IGF1 levels increase with age in normal children, and the same correlation could be found in these children. Although the mean IGF1 was within the normal range, 8 of the 33 children had levels (converted to SDS) below the normal range. IGF1 SDS at the start

of treatment correlated with height velocity SDS in CRF, and with height velocity in the transplanted children (Fig. 5). IGF1 SDS rose significantly with rhGH therapy in both groups (Table 5).

Administration of rhGH may change the ratio of IGFBP3 to IGF1, thus altering the proportion of free IGF1 and increasing its bioavailability.

Conclusion

Possible mechanisms of poor growth in CRF are decreased receptor cell responses to GH and IGF1. The causes remain unknown. It is possible that the increased circulating IGFBP3 fragments found in transplant patients may be a result of steroid therapy. These fragments may interfere with the bioactivity of IGF1. High basal GH levels with loss of GH pulsatility are caused by poor renal clearance, but may also, along with increased hypothalamic sensitivity, be driven by the decreased activity of the receptor cells.

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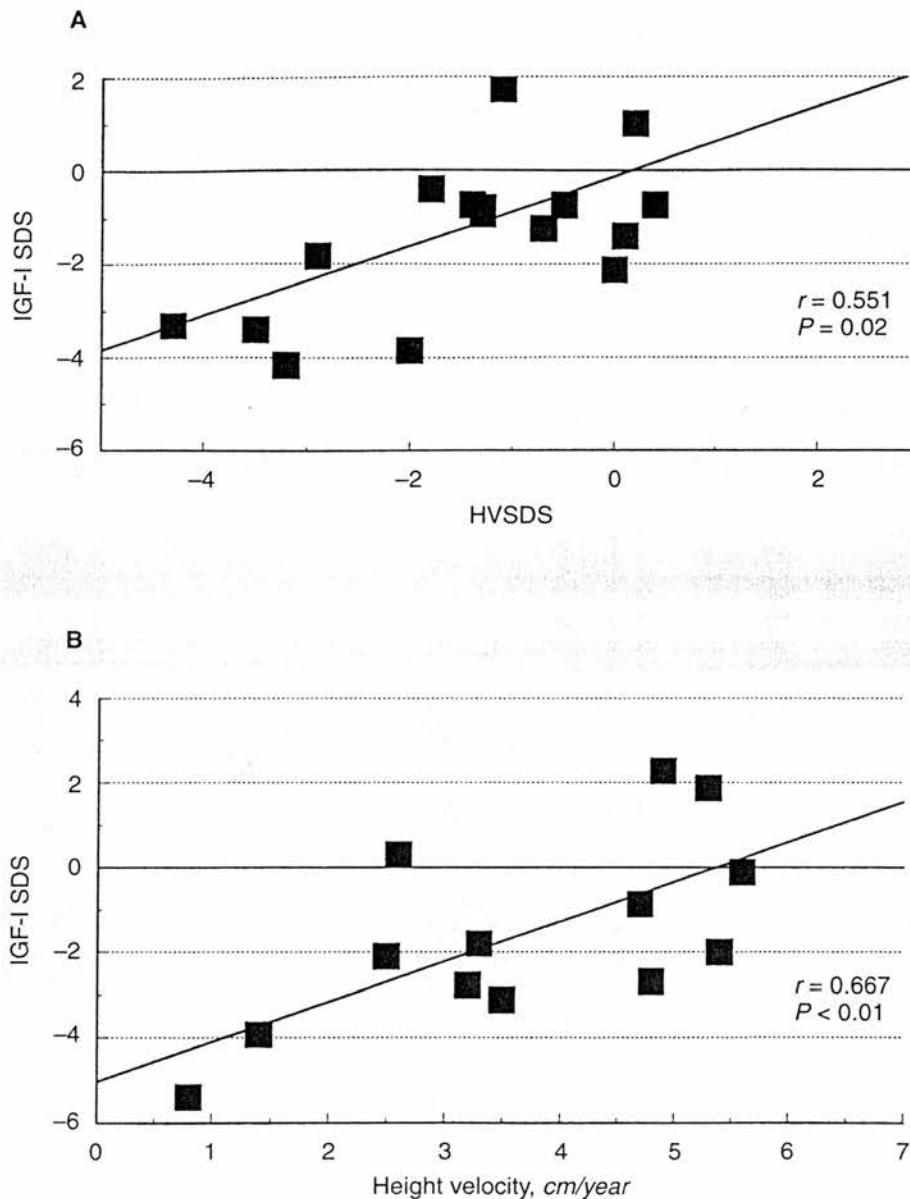


Fig. 5. Correlation between IGF-I SDS and growth over the previous year in CRF (A) and after transplantation (B).

Table 5. IGF-I SDS during rhGH treatment

Group	Day 1	6 Months	1 Year
IGF-I SDS			
CRF	-1.06 (1.89)	3.60 (3.01) ^a	3.63 (2.94) ^a
Transplant	-1.11 (2.00)	6.70 (4.32) ^a	5.52 (3.02) ^a

Results as mean and SD.

^a $P < 0.001$

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Catch-up growth occurs after renal transplantation in children of pubertal age

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Objective: Assessment of growth after renal transplantation in children of pubertal age by analyzing the annual increment in height standard deviation score (Ht SDS) in all girls ≥ 10 years and boys ≥ 11 years of age at the time of transplantation until latest follow-up (minimum 2 years).

Patients: A total of 59 grafts were placed in 54 recipients (30 boys) between December 1984 and January 1995. Mean (range) age at transplantation was 13.6 years (10.1 to 17.7 years). Fifty-one percent had congenital renal disease, 36% acquired renal disease, and 13% had hereditary nephropathies. Eighty-seven percent were first grafts; of these, 29% were performed pre-emptively, and 23% were from living related donors.

Results: Mean (SD) Ht SDS at transplantation was -1.8 (0.2) and increased significantly thereafter, such that it was -1.6 (0.2) at 1 year, $n = 52$; -1.5 (0.2) at 2 years, $n = 47$; -1.0 (0.2) at 3 years, $n = 27$; -0.7 (0.3) at 4 years, $n = 19$; and -0.6 (0.3), $n = 13$, at 5 years after transplantation (analysis of variance, $P < .001$). The greatest improvement in Ht SDS in the first year was seen in children with the highest glomerular filtration rate ($r = 0.429$, $P = .002$) and in those who were shortest at the time of transplantation ($r = -0.356$, $P = .009$).

Conclusion: Catch-up growth occurs in children receiving renal transplants during the expected time of puberty. (*J Pediatr* 1998;133:435-40.)

Chronic renal failure frequently results in poor growth, so that many children are short at the time of transplantation.^{1,2} Good catch-up growth after transplantation is related to a number of factors: young age,³⁻⁹ good graft function,^{6,7,9,10} significant growth delay at the time of transplantation,^{9,11,12} and the use of low doses of steroids,¹³ particularly when given on alternate days.^{12,14}

It has been stressed in several studies that although catch-up growth can be dramatic in young children, it rarely occurs in children who undergo transplantation during the pubertal age range.³⁻⁹ However, patients of pubertal age in our center were observed to have catch-up growth after transplantation comparable to that reported in younger children. Thus this study was designed to assess the growth of all children of pubertal age (girls aged ≥ 10 years and boys ≥ 11 years) at the time of transplantation who had received a renal transplant in our center since its inception 13 years ago. We also looked to see whether other factors known to influence post-transplant growth were affecting the growth of our patients.

METHODS

One hundred thirty-nine renal transplants were performed in 122 children over the 10-year period after the initiation of the transplant program in this center in December 1984. Fifty-nine of these grafts (in 54 recipients [30 boys]) were placed in children who were at or above the expected age for the onset of puberty (girls ≥ 10 years, boys ≥ 11 years). Data from these children are reported.

GFR	Glomerular filtration rate
Ht SDS	Height standard deviation score
NAPRTCS	North American Pediatric Renal Transplant Cooperative Study

From 1992 onwards, data were collected prospectively; before 1992, data were collected from review of the case notes. Age, diagnosis, treatment modality, number of human leukocyte antigen mismatches between donor and recipient, and height at the time of transplantation were noted. After transplantation, height and GFR by clearance of chromium ethylenediaminetetraacetic acid were measured after 6 months, 1 year, and then yearly. Prednisolone dose adjusted for surface area was noted at the end of each post-transplant year.

Immunosuppression consisted of azathioprine (60 mg/m^2), cyclosporin A (dose adjusted to maintain serum levels of 50 to 150 ng/mL), and prednisolone. Steroid equivalent to prednisolone $60 \text{ mg/m}^2/\text{day}$ divided into 2 doses was given intravenously initially, then orally when tolerated. This dose was tapered at intervals of 3 to 4 days to a dose of $10 \text{ mg/m}^2/\text{day}$ by 4 to 6 weeks after transplantation. Over the next 4 weeks the dose was reduced at approximately

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Table I. Growth data for 5 years after renal transplantation

Time from transplantation	Height SDS	No.	Increase in HtSDS from previous year	Cumulative increase in HtSDS
Day 1	-1.8 (-5.5 to 1.4)	52	0	0
1 Year	-1.6 (-5.0 to 1.1) *	52	0.19 (0.07)	0.19 (0.07)
2 Years	-1.5 (-4.5 to 1.2) *	47	0.10 (0.07)	0.35 (0.12)
3 Years	-1.0 (-4.5 to 1.8) *	27	0.16 (0.08)	0.67 (0.20) [†]
4 Years	-0.7 (-2.5 to 1.9) *	19	0.15 (0.08)	0.91 (0.24) [‡]
5 Years	-0.6 (-2.3 to 2.0) *	13	0.19 (0.07)	1.15 (0.31) [§]

Results are given as the mean (range) or mean (SE).

* $P < .01$ vs day 1.

[†] $P = .047$ vs increase in HtSDS after 2 years.

[‡] $P = .057$ vs increase in HtSDS after 3 years.

[§] $P = .02$ vs increase in HtSDS after 4 years.

weekly intervals to 5 mg/m²/day and then gradually converted, again at weekly intervals, to a dose of 10 mg/m² on alternate days by 10 to 12 weeks after transplantation. One year after transplantation the mean (SE) dose of prednisolone was 8.5 (0.6) mg/m² on alternate days; after 2 years 7.8 (0.5); after 3 years 7.5 (0.5); after 4 years 7.6 (0.6); and after 5 years 7.0 (0.6) mg/m² on alternate days.

Rejection episodes in the first 6 weeks after transplantation were treated with intravenous methylprednisolone (600 mg/m² daily for 3 days); thereafter treatment consisted of oral prednisolone (3 mg/kg daily for 3 days). Steroid-resistant rejection was treated with antithymocyte globulin (Merieux, France).

Of the 54 children, 51% had congenital renal disease, 36% had acquired renal disease, and 13% had hereditary nephropathies. Fifty-one of the 59 grafts were first grafts, 6 were second, and 2 were third. Of first grafts, 29% were pre-emptive transplants, and 23% were from living related donors. One of the second grafts was from a living related donor. The mean age at transplantation was 13.6 years (range 10.1 to 17.7 years). Only one child received recombinant human growth hormone after transplantation. Only growth data for the 18 months before recombinant human growth hormone was started is included for this child. Three children received recombinant human growth hormone before transplantation.

Pubertal staging data were available for those children who received transplants

from 1990 onwards (37 children). Of these children, 19 of 37 were pubertal (Tanner stage 2 or greater) at the time of transplantation, and 18 were prepubertal (Tanner stage 1). Nine of the 18 prepubertal children progressed into puberty during the study period after transplantation.

Growth data for 1 or more years was available for 51 children (52 grafts); 3 grafts were lost before 9 months, growth details were unavailable for 3 of the first grafts in patients who subsequently underwent retransplantation, and 1 child moved from the area. Correlation between the increase in Ht SDS during the first post-transplant year and age, Ht SDS at transplantation, pubertal stage, GFR, prednisolone dose, donor source, number of human leukocyte antigen mismatches, and number of rejection episodes was performed.

ANOVA was used to compare data from the same children at different time points. A paired Student *t* test was used to compare the same groups at 2 different times; an unpaired *t* test was used to compare 2 different groups. A *P* value of $< .05$ was considered significant.

RESULTS

One-year patient and graft survival rates were 100% and 93%, respectively; 5-year patient and graft survival rates were 98% and 75%, respectively.

Growth

One-year growth data were available for 51 patients (52 grafts). Mean Ht

SDS increased from -1.8 (-5.5 to 1.4) before transplantation to -1.6 (-5.0 to 1.1) after 1 year, $P = .007$. Ht SDS after each year of the study is given in Table I.

Individual patients' Ht SDS at yearly intervals after transplantation is shown in Fig 1, *A* for boys and Fig 1, *B* for girls.

GFR

The mean GFR was 53 (20 to 103) mL/min/1.73 m² at 6 months, 52 (12 to 103) at 1 year, 45 (12 to 89) at 2 years, 44 (19 to 75) at 3 years, 42 (26 to 63) at 4 years, and 48 (28 to 81) at 5 years. These values did not differ significantly.

Predictors of Growth Rate in the First Post-transplant Year

The greatest increase in Ht SDS during the first post-transplant year was seen in those children with the best renal function ($r = 0.429$, $P = .002$) (Fig 2). Increase in Ht SDS was also related to Ht SDS at transplantation ($r = -0.356$, $P = .009$) (Fig 3) but not to age at transplantation ($r = 0.217$, $P = .12$), dose of prednisolone ($r = -0.080$, $P = .59$), or pubertal stage ($r = 0.095$, $P = .60$). Increase in Ht SDS in the first post-transplant year was also unrelated to pre-emptive transplantation, donor source, degree of human leukocyte antigen mismatching, and number of rejection episodes.

The strongest predictor of increase in Ht SDS in the first year was GFR (mL/min/1.73 m²) at 1 year. Two factors were related to GFR. One was the number of rejection episodes; children with 2 or fewer rejection episodes had a higher

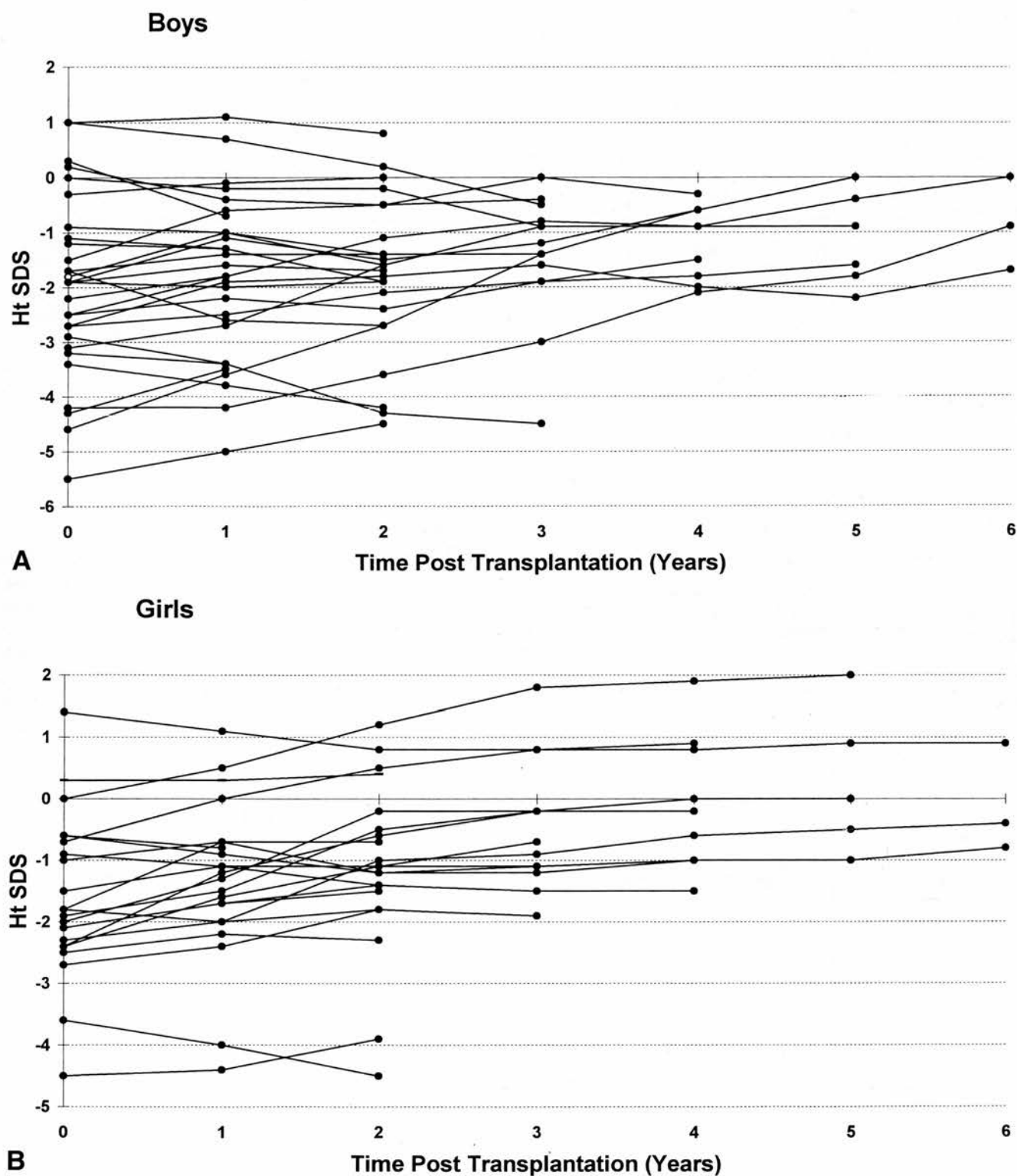


Fig 1. Individual patients' Ht SDS at yearly intervals for boys (A) and girls (B).

GFR than those with 3 or more rejection episodes in the first post-transplant year (63.8 [18.2] vs 46.6 [19.0], $P = .003$). The second factor was treatment modality be-

fore transplantation; children who had received a pre-emptive renal transplant had a higher GFR than those children who were receiving dialysis at the time of trans-

plantation (64.4 [20.3] vs 47.0 [21.2], $P = .015$). GFR was unrelated to donor status.

During the second post-transplant year there was a trend for increase in

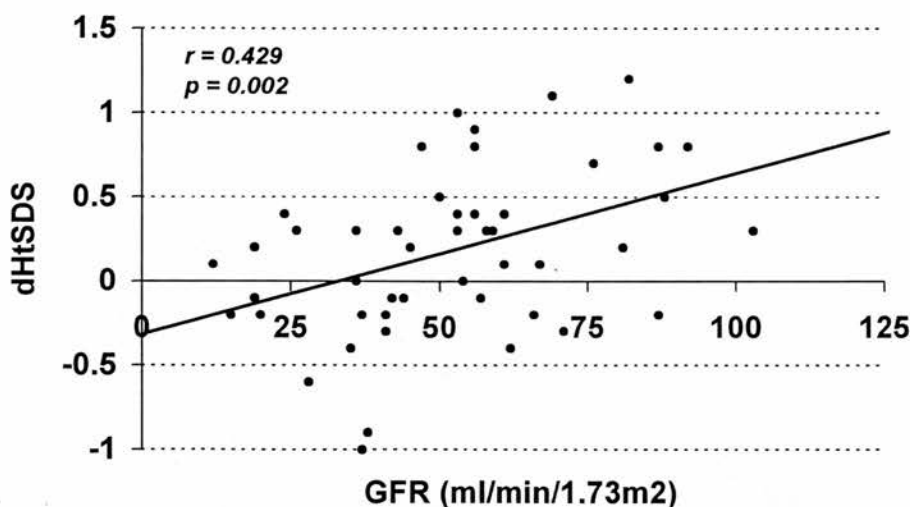


Fig 2. Increase in Ht SDS in first post-transplant year plotted against GFR at 1 year: Increase in Ht SDS was positively related to GFR at time of transplantation.

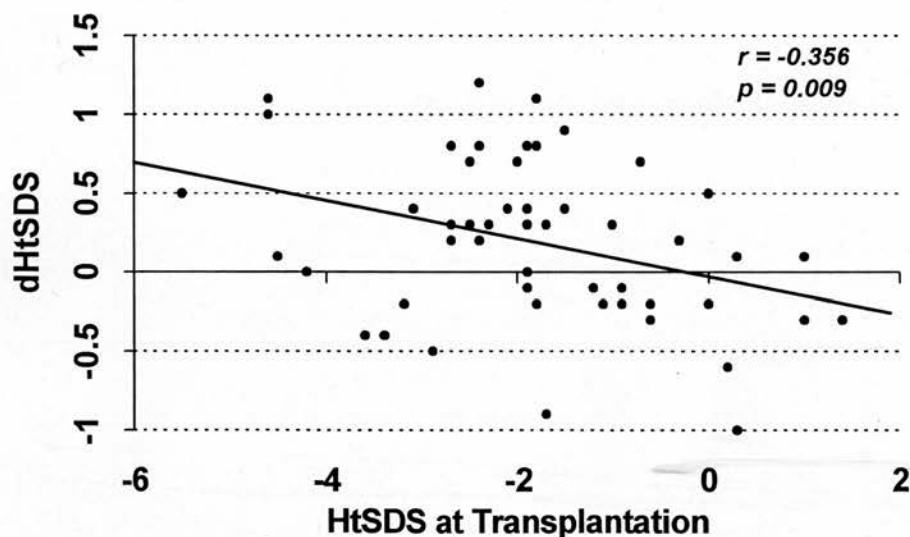


Fig 3. Increase in Ht SDS in first post-transplant year plotted against Ht SDS at time of transplantation. Children who were most stunted had greatest increase in Ht SDS.

Ht SDS to be greater in those children who had received a pre-emptive renal transplant (0.33 [0.14] vs 0.06 [0.08] SD, $P = .06$) and those with a higher GFR at 1 year ($P = .07$). In the third post-transplant year increase in Ht SDS was greatest in older children ($P = .05$) and in those who had received a pre-emptive transplant (0.36 [0.15] vs 0.04 [0.08] SD, $P = .06$).

DISCUSSION

We have demonstrated that significant catch-up growth can occur after renal

transplantation in children of pubertal age. Indeed, Ht SDS increased significantly for up to 5 years, by which time nearly all of the children had a height within the normal range.

Such improvement in height after transplantation is contrary to published data in children of this age.³⁻⁹ The large American database, North American Pediatric Renal Transplant Cooperative Study, describes catch-up growth for up to 2 years after transplantation in children <6 years but little or no change in Ht SDS in 6- to 13-year-olds and a decrease in Ht SDS in children 13 to 18 years old.⁹ Only 6% of 13- to 18-year-olds had

catch-up growth after 2 years, and after 5 years the proportion was little changed at 9%.¹⁵

The reports mentioned previously have studied children of slightly different age ranges.³⁻⁹ We chose age ranges of greater than or equal to 10 years in girls and 11 years in boys to ensure inclusion of all children going through puberty; in fact, 76% of children were in or entered puberty during follow-up. The NAPRTCS group used different age ranges for growth analysis⁹; if we include only children aged 13 years or over, so as to allow comparison with NAPRTCS, the mean (SE) increase in Ht SDS after 2 years in our cohort is 0.57 (0.16), $n = 28$, compared with -0.21 (0.08) for NAPRTCS and 1.1 (0.28), $n = 15$, after 3 years compared with -0.29 (0.13) for NAPRTCS.

It is not clear why our adolescents are growing so well. Factors known to affect post-transplant growth positively are young age³⁻⁹ and Ht SDS at the time of transplantation, with the most stunted showing the best response.^{9,11,12} However, the mean (SE) Ht SDS at transplantation was -1.9 (0.19) in our patients, which is similar to that in other studies: NAPRTCS -2.41 (0.09),⁹ -1.62,⁶ -2.6 (0.40),¹¹ and -2.41.¹²

Growth after transplantation is negatively affected by declining graft function.^{6,7,9,10} Publications from the NAPRTCS database quote mean creatinine clearance values of 68 and 65 mL/min/1.73 m² for living related donor and cadaveric grafts after 1 year in children >12 years at the time of transplantation.¹⁶ The mean GFR in all of our patients after 1 year was 52 mL/min/1.73 m² (20-103) as measured by the clearance of chromium ethylenediamine tetraacetic acid. Creatinine clearance can overestimate GFR and may explain the higher values in NAPRTCS. However, it is clear that our results cannot be explained on the basis of better graft function.

A possible explanation for our results is the early use of low-dose steroid therapy. Growth after transplantation is reported to be influenced by total dose of steroid,¹³ although no such correlation was found in this study. This may be due to the narrow dose range used or perhaps could be explained by a recent re-

port that was able to relate growth after renal transplantation to exposure to prednisone (area under the curve) but not to the dose per kilogram.¹⁷

In addition to using a low dose of steroids, we also favor early conversion to an alternate day regimen. The same total dose of steroid given on alternate days is less growth-suppressive than when given daily for juvenile chronic arthritis,¹⁸ ulcerative colitis, asthma, and nephrotic syndrome.¹⁹ In children with renal transplants there are uncontrolled reports of normal^{12,20} and poor growth^{11,21,22} with prednisolone doses of 30 mg/m² or less on alternate days. Broyer et al.¹⁴ compared 17 children who were changed from daily to alternate day prednisone 1 to 2 years after transplantation, with 18 who remained on the same total dose given daily. Ht SDS increased by 0.5 SD in the alternate day group but decreased by 0.1 SD in the daily group.

In our patients prednisolone was tapered to 10 mg/m² on alternate days by 12 weeks after transplantation. The NAPRTCS database reports doses of prednisone of 0.31 mg/kg/day 6 months after transplantation and 0.19 mg/kg/day after 30 months.²³ Although these total doses are not dissimilar to ours, the difference is that only 12% to 33% of the NAPRTCS children were treated with alternate day steroids. In other studies in which older children showed poor growth, most received daily steroids in relatively high dose^{5,7}; when children received alternate day steroids, they received a high dose.^{4,10} The NAPRTCS database has also reported improved growth in those children receiving alternate day steroids.²⁴

The reason for the continuing use of daily steroids is the fear of transplant rejection. Although a number of studies describe the benefits of alternate day steroids or steroid withdrawal on growth,²⁵⁻²⁷ many report an increased incidence of rejection.²⁷⁻²⁹ Acute rejection rates of between 39% and 56% have been described after stopping steroids,^{28,29} and rates of up to 30% after conversion to alternate day regimens have been reported.^{12,14,20,27} In the study by Broyer et al.,¹⁴ no increase occurred in the number of rejection episodes in the group con-

verted to alternate day steroids. Although we do not have control data with which to compare our rejection rate, our overall graft survival data are no different from those of other published series,¹⁶ and GFR at 5 years is well maintained. Therefore good growth in our patients is not at the expense of loss of graft function.

GFR in the first year after transplantation was greater in those children who had received a pre-emptive transplant; the difference was more than could be explained by native renal function. Increase in Ht SDS in the second and third years after transplantation was greater in patients who had received pre-emptive transplants. The number of pre-emptive transplants performed in our center may possibly have a bearing on our results.

In conclusion, we have shown that renal transplantation during the pubertal age range can result in improved growth for up to 5 years. Catch-up growth at this age is contrary to published data, and we postulate that it may be related to the consistent and early use of alternate day prednisolone. Such a steroid regimen was not associated with increased graft loss nor with a poor prediction for GFR at 5 years in our patients.

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Randomised controlled trial of recombinant human growth hormone in prepubertal and pubertal renal transplant recipients

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Abstract

Aims—To evaluate the efficacy (height velocity (HV), change in height standard deviation score (Δ HSDS)), and safety (glomerular filtration rate (GFR), incidence of rejection, and calcium and glucose metabolism) of recombinant human growth hormone (rhGH) treatment after renal transplantation.

Design—A two year randomised controlled trial.

Subjects—Fifteen prepubertal and seven pubertal children: mean (SD) age, 13.0 (2.6) and 15.2 (2.4) years, respectively; mean (SD) GFR, 51 (30) and 48 (17) ml/min/1.73 m², respectively. Six prepubertal and three pubertal children were controls during the first year; all received rhGH in the second year.

Results—In the first year, mean (SE) HV and Δ HSDS in the prepubertal treated group increased compared with controls: 8.1 (0.9) v 3.7 (0.6) cm/year and 0.6 (0.1) v -0.3 (0.2), respectively. In the pubertal treated group, mean (SE) HV and Δ HSDS were also greater: 10.1 (0.6) v 3.9 (1.3) cm/year and 0.6 (0.1) v -0.1 (0.2), respectively. Comparing all treated and control children, there was no significant change in GFR: treated group, mean (SE) 9.9 (5.4) ml/min/1.73 m² v control group, -1.6 (7.6) ml/min/1.73 m². There were also no differences in the incidence of rejection in the first year: eight episodes in 13 patients v five episodes in nine patients, respectively. Phosphate, alkaline phosphatase (ALP), parathyroid hormone (PTH), and fasting insulin concentrations rose during the first year of treatment, but not thereafter. In the second year of treatment, HV remained above baseline.

Conclusion—Treatment with rhGH improves growth in prepubertal and pubertal children with renal transplants, with no significant change in GFR or the incidence of rejection. Phosphate, ALP, PTH, and insulin increased during the first year of treatment.

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Keywords: recombinant human growth hormone; growth; renal transplantation; renal function; transplant rejection

Recombinant human growth hormone (rhGH) improves short term growth in children with chronic renal failure,^{1,2} and has been shown in

preliminary studies to improve growth in children with renal transplants.¹⁻⁵ We report a two year, prospective controlled trial of rhGH in children after renal transplantation.

The use of rhGH after transplantation requires close monitoring. There is potential for rhGH to have an adverse effect on: renal function,⁶ the incidence of transplant rejection,⁷ renal bone disease,⁸ and glucose metabolism.⁹ In view of this, glomerular filtration rate (GFR), rejection rate, and indices of calcium and glucose metabolism were followed in our study.

Our study group included both prepubertal and pubertal children, and the growth data for these two groups are reported separately. Some of the children remained on rhGH treatment after the trial was complete and data from these children are also reported.

Methods

Twenty two children with renal transplants were enrolled into a two year, open labelled, prospective and controlled trial of the use of rhGH. Patients from three paediatric nephrology centres in the UK were stratified according to pubertal stage, and then randomised to rhGH (treatment group) or no treatment (control group) in the first year of the trial, with all children receiving rhGH in the second year. Six children continued on rhGH treatment after completion of the trial; data from these children are also reported (follow up data). Ethical approval was obtained from the centres involved, and written consent was obtained from the parents.

The children were seen on day 1, day 8, and then at three monthly intervals for two years. On each occasion, height, weight, blood pressure, and blood biochemistry including urea, creatinine, calcium, phosphate, alkaline phosphatase (ALP), intact parathyroid hormone (PTH), glucose, insulin, and glycosylated haemoglobin (HbA1c) were checked. The growth standards of Tanner and Whitehouse¹⁰ were used to calculate height standard deviation score (HSDS). Per cent ideal weight for height (WFH) was calculated six monthly.

For the duration of the trial, the occurrence of adverse events was noted. Every six months, GFR was measured by clearance of inulin, using a constant infusion and urine collections.² A rejection episode was defined as an episode of transplant dysfunction treated with three days of high dose oral prednisolone. The diagnosis was made by the managing clinician.

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Table 1 Height velocity and height velocity SDS for the treatment and control groups

Patients	Height velocity (cm/year)			Height velocity SDS		
	-1 Year	Year 1	Year 2	-1 Year	Year 1	Year 2
Prepubertal						
Treatment group (n = 9)	3.9 (1.7)	8.1 (2.7)	5.7 (2.9)†	-0.8 (1.8)	5.3 (5.0)	3.0 (5.8)†
Control group (n = 6)	4.9 (1.8)	3.7 (1.5)	8.5 (1.6)†	-1.1 (3.0)	-1.6 (2.1)	3.0 (4.6)*
		p < 0.005			p < 0.01	
Pubertal						
Treatment group (n = 4)	4.5 (2.5)	10.1 (1.2)	6.4 (2.1)	-2.8 (2.1)	5.5 (3.9)	3.6 (1.9)
Control group (n = 3)	2.7 (1.6)	3.9 (2.3)	6.1 (4.7)	-3.0 (2.6)	-0.2 (1.0)	4.9 (5.9)
		p < 0.005			p = 0.06	

Values are mean (SD). *p < 0.05; †p < 0.005 compared with the previous year.

Recombinant human growth hormone (Genotropin; Pharmacia and Upjohn, Stockholm, Sweden) at a dose of 0.14 IU (0.05 mg)/kg/day (equivalent to a dose of 4 IU/m²/day) was given as a subcutaneous injection each evening by the child or a parent.

INCLUSION CRITERIA

Children were considered eligible for entry to the trial if they fulfilled the following criteria: height less than the third centile for age and sex or a height velocity (HV) < 25th centile; normal thyroid function. All had been transplanted for at least one year, and had a minimum calculated GFR of 20 ml/min/1.73m².¹¹

EXCLUSION CRITERIA

Children were excluded from the trial for the following reasons: HV > 75th centile during the preceding six months, treatment with any form of growth hormone in the past year, a previous malignancy, a severe congenital abnormality, diabetes mellitus, or uncontrolled renal bone disease.

The prepubertal group was defined by a testicular volume of < 4 ml in boys and breast development less than Tanner stage B2 in girls.¹² Children who were in the early stages of puberty were enrolled into the pubertal group (testicular volume ≥ 4 ml and ≤ 10 ml, breast stage B2 or B3).

PATIENTS

Fifteen prepubertal (two girls) and seven pubertal patients (two girls) were enrolled and randomised to receive either rhGH or no rhGH in the first year. Nine prepubertal and four pubertal children received rhGH in the first year (treatment group); all 22 children received rhGH in the second year.

In the prepubertal group, the mean age was 13.0 (range, 9.4–16.5) years and mean GFR was 51 (range, 13–117) ml/min/1.73m². The respective values in the pubertal group were 15.2 (range, 12.4–19.8) years and 48 (range,

23–78) ml/min/1.73m². Eleven of the prepubertal children had renal failure secondary to a congenital structural problem, two had glomerulonephritis, one cystinosis, and the other had atypical haemolytic uraemic syndrome. In the pubertal group, three had congenital structural problems, two glomerulonephritis, one congenital nephrotic syndrome, and one had haemolytic uraemic syndrome. HSDS at baseline, and HV and height velocity standard deviation score (HVS DS) during the preceding year are given in tables 1 and 2. Immunosuppression consisted of azathioprine (60 mg/m²), prednisolone (10 mg/m² on alternate days), and cyclosporin A. Cyclosporin A dose was adjusted to maintain plasma trough levels between 50 and 150 ng/ml.

FOLLOW UP DATA

Fourteen children have been followed up for two years, three for three years, two for five years, and one child has been treated for six years. HV and calculated GFR were measured during this time. In addition, the first year of rhGH treatment in all patients was compared with the growth rate during the preceding year. Age, pretreatment HV and HSDS, GFR, and steroid dose were correlated with the increase in HV and HSDS during the first year of treatment.

STATISTICAL ANALYSIS

Results are expressed as mean (range) or mean (SD). Within and between group results were compared using the paired or unpaired Student's *t* test, respectively. Comparison of change within the treatment and control groups was made by calculating and comparing the mean (SE) change. Frequencies were compared using the χ^2 test. Analysis of multiple results within the same group over time was performed by analysis of variance (ANOVA). Correlations were performed using Pearson's correlation coefficient, and regression by both single and multiple linear regression analyses. Significance was set at a *p* value of < 0.05.

Results

TREATMENT GROUP (NINE PREPUBERTAL AND FOUR PUBERTAL CHILDREN)

Two prepubertal children stopped rhGH; one child returned to dialysis after 11 months, the other developed glucose intolerance after nine months of treatment. Two children stopped treatment during the second year; one prepu-

Table 2 Height SDS at yearly intervals for the treatment and control groups

	-1 Year	Day 1	Year 1	Year 2
Prepubertal				
Treatment group (n = 9)	-3.4 (0.7)	-3.6 (1.0)	-3.0 (1.0)†	-2.6 (1.3)*
Control group (n = 6)	-3.0 (0.7)	-3.0 (0.8)	-3.3 (1.0)	-2.8 (1.0)‡
Pubertal				
Treatment group (n = 4)	-2.1 (1.2)	-2.4 (1.4)	-1.9 (1.3)*	-0.9 (0.4)
Control group (n = 3)	-2.3 (0.1)	-2.6 (0.2)	-2.7 (0.5)	-2.3 (0.8)

Values are mean (SD).

*p = 0.02 compared with day 1; †p = 0.001 compared with day 1; ‡p = 0.02 compared with year 1, within the same groups.

bertal child had started the trial with poor graft function and received a second graft after 18 months of rhGH, the other pubertal child had an increase in creatinine.

CONTROL GROUP (SIX PREPUBERTAL AND THREE PUBERTAL CHILDREN)

One pubertal child was withdrawn after 18 months because of a poor response to six months of rhGH treatment.

GROWTH

Prepubertal group (n = 15)

During the first year of the study, HV and HVSDS were greater in the treatment group than the control group (table 1). The mean (SE) increase in HSDS (Δ HSDS) during the first year of study was 0.6 (0.1) in the treatment group and -0.3 (0.2) in the control group; $p < 0.001$ (table 2).

In the treatment group, there was no difference between Δ HSDS in the first and second years of treatment; 0.6 (0.1) v 0.4 (0.2), respectively ($p = 0.34$). HV and HVSDS were lower in the second year compared with the first year of treatment (table 1). The increase in mean (SE) HSDS during the second year in the treatment group was greater than that in the first year in the control group (0.4 (0.2) v -0.3 (0.2); $p = 0.003$), but HV and HVSDS were not significantly different ($p = 0.16$ and 0.09, respectively). In the control group, HV, HVSDS, and Δ HSDS were greater in the second year of rhGH treatment than in the first year (tables 1 and 2).

Pubertal group (n = 7)

The results in the pubertal group were similar to those in the prepubertal group; however, the numbers of children in the treatment and control groups were small. During the first year of the trial, HV and Δ HSDS were significantly greater in the treatment group than the control group; the corresponding p value for HVSDS was $p = 0.06$ (tables 1 and 2).

In the treatment group, HV, HVSDS, and Δ HSDS remained above pretreatment values in the second year of the study, but were lower than the respective values in the first year of the trial. Comparing the first and second year data in the control group, HV, HVSDS, and Δ HSDS were greater in the second year than in the first year, but the values were not significant (tables 1 and 2).

Pooled follow up data for all prepubertal children during rhGH treatment

When data are pooled for all 15 prepubertal children during their first year of rhGH treatment, mean HV increased significantly from 3.8 (range, 0.8–6.9) cm/year in the year before treatment to 8.2 (range, 3.0–12.5) cm/year; $p < 0.001$. The corresponding values for HVSDS were -1.1 (range, -4.7 to 2.1) and 4.4 (range, -2.7 to 13.6; $p < 0.001$). Mean HSDS decreased from -3.2 (range, -5.1 to -2.0) to -3.5 (-5.8 to -1.9; $p = 0.015$) in the year before treatment, and subsequently rose to -2.9 (-5.0 to -1.3) after one year of treatment ($p < 0.001$).

Ten prepubertal children received rhGH for at least two years. In these children, HSDS increased from -3.4 (-5.8 to -1.9) on day 1, to -2.4 (-4.4 to -1.2) after two years ($p = 0.002$). Mean HV was 3.9 (range, 0.8–6.9) cm/year in the year before treatment, 8.2 (range, 3.0–10.7) cm/year in the first year ($p < 0.0002$ compared with the previous year), and 6.1 (range, 0.1–8.8) cm/year during the second year ($p = 0.012$ compared with the year before treatment). The mean gain in height was 14.8 (range, 3.1–18.5) cm in two years. Three children received three years of rhGH treatment; mean height gain was 22.1 (range, 21.8–22.4) cm in three years. One child received rhGH for five years and another for six years; HVSDS remained above pretreatment values during this time.

Pooled follow up data for all pubertal children during rhGH treatment

Considering all seven pubertal children, mean HV increased from 4.2 (range, 1.3–8.1) cm/year in the year before treatment to 8.4 (range, 1.8–11.7) cm/year ($p < 0.05$) during the first year of treatment. Mean HSDS was -2.3 (range, -3.8 to -1.2) one year before starting treatment, -2.5 (range, -4.5 to -1.6) at the start of treatment, and -2.0 (range, -3.7 to -0.9) after one year ($p < 0.05$). Five of the seven patients completed the study, but elected to stop treatment. Four children received two years of rhGH: mean HV was 5.2 (range, 3.5–8.1) cm/year before treatment, 9.3 (range, 5.3–11.7) cm/year in the first year of treatment, and 5.8 (range, 4.1–8.7) cm/year in the second year of treatment, values very similar to the prepubertal group. The average height gain during two years of rhGH was 15.0 (range, 9.5–19.3) cm.

Regression analysis

There was no significant difference in the increase in HSDS and HV in the first year of treatment between the prepubertal and pubertal groups. Combining the prepubertal and pubertal groups, HV before and during rhGH was related to age ($r = -0.417$; $p = 0.05$ and $r = -0.463$; $p = 0.03$, respectively). Multiple regression analysis revealed that prednisolone dose ($p = 0.028$) and age ($p = 0.049$) were the strongest negative predictors of Δ HSDS. Using single regression analysis, HV during treatment was positively correlated with GFR ($r = 0.429$; $p = 0.016$), but this relation became non-significant when the effects of age and prednisolone dose were taken into account. There was no significant association between Δ HSDS and HSDS on day 1 ($r = 0.034$) or between Δ HSDS and HV ($r = 0.265$) before treatment.

Weight for height

At the start of treatment, all children had a WFH $> 100\%$. The two lowest values (105% and 108%) were in the two children who received no prednisolone treatment. Mean WFH decreased during the first year of treatment from 133% (range, 105–171%) at the start of treatment, to 125% (range,

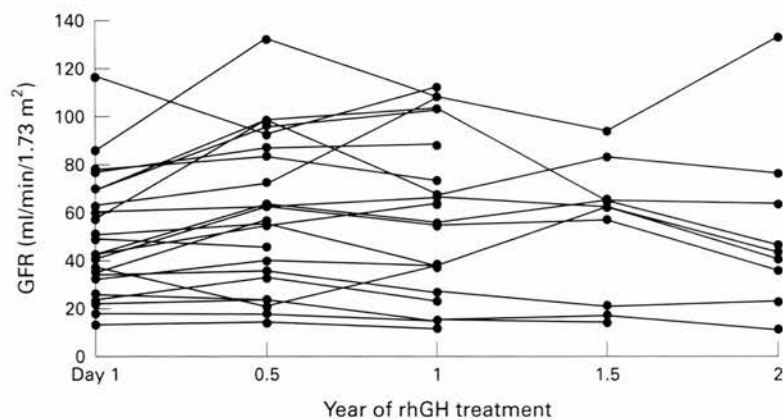


Figure 1 Glomerular filtration rate (GFR) as measured by inulin clearance in individual patients at six monthly intervals during the first two years of rhGH treatment.

99–152%) at six months ($p < 0.001$), and 122% (range, 96–152%) at one year ($p < 0.001$). WFH remained significantly reduced after two years 120% (range, 88–168%; $p = 0.016$).

RENAL FUNCTION

Results for the prepubertal and pubertal children were combined; GFR at baseline was comparable in the treatment and control groups ($p = 0.12$). The mean (SE) change in GFR during the first year was 9.9 (5.4) ml/min/1.73m² in the treated group ($n = 12$) and -1.6 (7.6) ml/min/1.73m² in the control group ($n = 9$; $p = 0.22$). One child in the treatment group was withdrawn during the first year because of graft failure. Setting his GFR to zero at the one year visit does not significantly alter the change in GFR during the first year: treated group mean (SE) GFR 7.2 (6.0) ml/min/1.73m² ($p = 0.36$).

Individual GFR values for all children during their first year of rhGH treatment are shown in fig 1. Mean GFR increased significantly during rhGH treatment: 49 (range, 13–117) ml/min/1.73m² on day 1 and 58 (range, 14–133) ml/min/1.73m² after six months ($n = 22$; $p = 0.01$). In those children who completed a full year of rhGH, GFR remained higher at one year; 51 v 59 ml/min/1.73m² ($n = 19$; $p = 0.04$). However, if a value of zero is included for the child who lost his graft, then the change in GFR becomes

non-significant ($n = 20$; $p = 0.12$). There was no further change in GFR during the second year of rhGH treatment. Calculated GFR in the children who continued on treatment after the study was complete did not change (data not shown).

Mean serum creatinine in the treated and control groups did not differ at baseline: control group, 100 (range, 49–152) μ mol/l and treated group, 114 (range, 46–220; $p = 0.46$). The mean (SE) change in creatinine during the first year appeared to be greater in the treated group (30.1 (11.7) μ mol/l) than in the control group (3.8 (6.2) μ mol/l), but was not significantly different ($p = 0.11$). Mean creatinine at one year in the treated group was 146 (range, 64–317) μ mol/l ($p = 0.013$ compared with day 1) and did not increase further during the second year of treatment; mean creatinine at two years was 146 (range, 70–402) μ mol/l. Urea decreased slightly after one week of rhGH treatment, but was unchanged thereafter (data not shown).

Presumed rejection episodes

In the first year of the trial there were eight presumed rejection episodes in 13 patients in the treatment group and in nine patients in the control group ($p > 0.10$). In the control group, there were no differences between the numbers of episodes in the first and second years of the trial; five episodes in nine patients in each year. Combining the data from all 22 children, there were 15 episodes in 22 patients in the first year of treatment, which was no different from the year before rhGH treatment (11 episodes in 22 patients; $p > 0.10$).

ADVERSE EVENTS

A 10 year old girl had raised fasting glucose, insulin, and HbA1c concentrations after nine months of rhGH treatment. These values returned to normal when rhGH was stopped. Several years before, while on dialysis, she had pancreatitis and required a partial pancreatectomy. Glucose tolerance tests before and after withdrawal from the trial were normal. She went on to develop insulin dependent diabetes, and chose to restart rhGH treatment once established on insulin replacement.

Table 3 Indices of calcium and glucose metabolism in the treatment and control groups

Control group	Normal range	Day 1	3 months	6 months	1 year		
Calcium (mmol/l)	2.10–2.60	2.43 (0.09)	2.45 (0.04)	2.44 (0.08)	2.46 (0.08)		
Phosphate (mmol/l)	0.8–1.50	1.23 (0.23)	1.29 (0.21)	1.20 (0.23)	1.32 (0.16)		
ALP (units/l)	130–520	231 (81)	212 (89)	240 (145)	214 (126)		
PTH (ng/l)	< 65	43.7 (35.3)	64.6 (48.1)	69.0 (46.0)	61.1 (44.9)		
Glucose (mmol/l)	2.5–5.3	5.0 (0.6)	4.7 (0.8)	5.0 (0.8)	5.0 (0.7)		
Insulin (units/l)	3–17	12.7 (6.1)	13.2 (4.8)	21.2 (19.8)	18.8 (15.2)		
HbA1c (%)	2.8–4.9	4.7 (0.9)	4.9 (1.1)	4.5 (1.2)	4.9 (0.8)		
Treatment group	Day 1	Day 8	3 months	6 months	1 year	18 months	2 years
Calcium (mmol/l)	2.39 (0.14)	2.42 (0.16)	2.40 (0.17)	2.41 (0.14)	2.51 (0.16)	2.43 (0.08)	2.40 (0.21)
Phosphate (mmol/l)	1.35 (0.39)	1.39 (0.35)	1.63 (0.33)**	1.55 (0.21)*	1.57 (0.27)*	1.49 (0.28)	1.44 (0.31)
ALP (units/l)	201 (80)	152 (44)	313 (89)**	305 (87)**	275 (77)**	236 (72)	273 (97)
PTH (ng/l)	38.1 (15.2)	37.1 (24.7)	50.8 (29.7)	58.1 (39.4)*	61.7 (35.5)	50.7 (24.9)	58.6 (63.6)
Glucose (mmol/l)	4.8 (0.9)	5.5 (0.7)	5.3 (0.8)	4.8 (0.6)	5.0 (1.2)	5.0 (0.7)	4.6 (0.6)
Insulin (units/l)	15.0 (7.8)	38.9 (30.7)*	36.6 (30.3)*	24.4 (12.4)*	18.1 (10.2)	36.2 (23.9)	18.9 (7.4)
HbA1c (%)	4.4 (0.8)	—	4.3 (1.0)	4.4 (0.9)	4.4 (1.1)	4.8 (0.8)	4.6 (0.8)

All values except normal ranges are mean (SD).

* $p < 0.05$, ** $p < 0.01$ compared with day 1.

A 13 year old boy in the control group developed worsening of a pre-existing idiopathic scoliosis during rhGH treatment, which required surgical correction the following year.

RENAL OSTEODYSTROPHY

Mean serum calcium, phosphate, ALP, and PTH are shown in table 3. Phosphate, ALP, and PTH increased significantly during the first year of rhGH treatment, but did not change significantly thereafter. No child developed overt renal osteodystrophy.

GLUCOSE

Glucose, insulin, and HbA1c values are shown in table 3. In the treatment group, there was a trend for glucose to increase after one week ($p = 0.10$), but not thereafter. When the results for all children during rhGH treatment were considered, there was a significant increase in glucose after one week: 4.8 (0.9) on day 1 and 5.4 (0.7) on day 8 ($p < 0.005$). Fasting insulin was raised in the treatment group after one week of rhGH treatment and remained so until six months, returning toward baseline thereafter. HbA1c was unchanged in the treatment group.

Discussion

This is one of the few controlled trials of rhGH treatment in children with renal transplants to date. This group of children were a subgroup of a large multicentre European study. We have established that rhGH improves growth in both prepubertal and pubertal patients with transplants, that the growth response was equal in the two groups, and that improved growth persisted during the second year of treatment. Comparing the control and treatment groups, there was no apparent adverse effect on graft function.

There are several possible mechanisms whereby rhGH or its mediator, insulin-like growth factor-I (IGF-I), might affect graft function: (1) rhGH increases renal plasma flow and GFR in adults with normal renal function^{6,13}; (2) there are known interactions between rhGH and the immune system, which could potentially cause an increase in the rate of rejection episodes¹⁴; and (3) rhGH could have a direct effect on the kidney, because there are growth hormone receptors on mesangial cells and IGF-I receptors on proximal tubular and mesangial cells.¹⁵ Because of the relation between rejection episodes and GFR, it is very difficult to study the effects of rhGH on these parameters in isolation.

We have reported previously that children with renal transplants respond to rhGH with a transient increase in GFR,¹⁶ similar to the normal adult kidney.⁶ In renal impairment, an increase in GFR might hasten the progression of decline in GFR, the so called hyperfiltration theory.¹⁷ There is no evidence that rhGH adversely affects GFR in children with chronic renal failure,^{2,18} but few studies of rhGH in renal transplantation have formally assessed GFR. Most studies report changes in creatinine or calculated GFR, and an analysis of

these trials has been inconclusive.¹⁹ Our data suggest that two years of rhGH treatment does not adversely effect graft function.

However, two children were withdrawn from the study because of a deterioration of renal function. For one patient there was suspicion of non-adherence to immunosuppression, while the other child had an acute rejection episode after six months of treatment. His GFR at one year was little changed from baseline, but one week later he had a further increase in creatinine and was withdrawn from the study. A third child received a second renal transplant after 18 months of rhGH. His renal function had shown a gradual deterioration before and during the trial. The small number of patients, the variability of the clinical course after transplantation, and the expected decline in graft function over time hamper interpretation of the data.

There was no apparent untoward effect of rhGH on the incidence of acute rejection. Some reports suggest that rhGH does not affect acute rejection,^{1,3,4,20} while others suggest that it does.^{7,21,22} Biopsy proven, acute rejection has been documented in several children at varying intervals after starting rhGH treatment.^{7,21,23,24} None of these were controlled trials. One study suggests that the risk of rejection is increased by rhGH, but only in children who have had rejection episodes before treatment.²² In the first year of rhGH treatment, seven of our patients had 15 presumed rejection episodes; all but one had suffered presumed rejection episodes in the previous year.

The incidence of rejection episodes in our study is higher than other studies. This is explained partly by the fact that these were presumed and not biopsy proven episodes and, therefore, likely to be an overestimation. Several patients had chronic rejection at the start of treatment, and other studies report increases in creatinine during rhGH when there is biopsy proven, chronic rejection.²⁴⁻²⁶ It is difficult to determine whether rhGH has decreased GFR, if there has been an increase in muscle bulk, or if this is the natural progression of chronic rejection. A recent study of mixed lymphocyte cultures using lymphocytes from paediatric renal transplant recipients and donor cells, showed that overall, the addition of rhGH to the culture had little effect, although three patients had an augmented response. These three patients had biopsy proven, chronic rejection.²⁷

The best response to rhGH was seen in the youngest children and in those on the least steroid, with the strongest predictor being the dose of prednisolone ($p = 0.029$). Growth in children with renal transplants, who are not on rhGH, has been correlated with age, GFR, and with steroid treatment.^{28,29} Children on alternate day steroids grow better than those on daily steroids,²⁹ and peak height velocity during puberty in patients with renal transplants is inversely correlated with steroid dose,³⁰ so that the relation between response to rhGH and steroid dose is not surprising. In another post-transplant study,²³ the change in HSDS was

greater than 0.4 after one year of rhGH treatment in five of five children not receiving steroids and one child on alternate day steroids, but was only -0.2 (range, -0.6 to 0.3) in 11 children on daily steroids. Mean creatinine was the same in both groups. All of our patients were receiving alternate day steroids.

Whether rhGH benefits final height, particularly if it is not started until puberty, has yet to be determined. In renal failure, puberty is delayed and the magnitude of the pubertal growth spurt is attenuated.³⁰ Puberty would seem to be an appropriate time to use rhGH, to induce or mimic the endogenous growth spurt, but there is concern that treatment might shorten the duration of the pubertal growth spurt.³¹ Some reports have suggested that this might be the case in children with renal transplants who receive rhGH,^{25, 32} while others have demonstrated a substantial increase in height during adolescence.^{3, 20} There was no undue advancement in bone age in our study (data not shown), nor in other reported studies.²⁰ The increase in HSDS was similar in the prepubertal and pubertal treatment groups in our study, with little or no change in HSDS in either of the control groups. However, longer follow up will be necessary to determine if there is a positive effect on final adult height.

ALP, serum phosphate, and PTH increased during treatment. rhGH increases phosphate reabsorption by the renal tubule, and the increase in serum phosphate stimulates PTH.³³ It is important that PTH is monitored regularly during rhGH treatment. There is a concern that rhGH treatment might be associated with an increase in the incidence of slipped capital femoral epiphyses, avascular necrosis of the femoral head, or worsening renal osteodystrophy in children with renal failure.⁸ Routine hip x rays were not performed in our study, but no child developed overt renal osteodystrophy.

One of the patients developed glucose intolerance during the study. This is a recognised complication of acromegaly.⁹ Patients with renal disease have peripheral resistance to the actions of insulin,³⁴ which is aggravated in patients who have received transplants by the use of corticosteroids.³⁵ Glucose intolerance during rhGH treatment after transplantation has been reported previously.²³ Our patient had undergone a partial pancreatectomy, which might have been a contributing factor. For the group as a whole, the increases in fasting glucose and insulin were transient, and during two years of rhGH treatment there was no increase in HbA1c. There would appear to be no longer term adverse effect on glucose tolerance.

In summary, rhGH improves short term growth in prepubertal and pubertal children with renal transplants compared with controls. Mean HV during the second year of rhGH treatment remained above the baseline value in the prepubertal group. In both groups, the approximate height gain was 15 cm after two years of rhGH treatment. There was no increase in the incidence of rejection episodes. There was a significant increase in GFR during

the first six months of treatment, but not thereafter. With continued use of rhGH, HVSDS reduces towards baseline, but growth can remain above pretreatment values for up to six years.

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BRIEF REPORT

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Growth hormone and markers of immune function in children with renal transplants

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Abstract Lymphocyte subsets and T cell activation markers were measured in ten children with renal transplants for up to 1 year before and during their 1st year of recombinant human growth hormone (rhGH) treatment. The number of lymphocytes, helper or cytotoxic T cells or natural killer cells, and the T cell expression of CD25, CD26 and HLA-DR antigens were not altered by rhGH. B cell numbers declined both before and during treatment. There was no difference in lymphocyte subset numbers between children with and without rejection episodes.

Key words Recombinant human growth hormone · Renal transplantation · Growth · Lymphocyte subsets · T cell activation markers

Introduction

The use of recombinant human growth hormone (rhGH) in transplanted children is controversial because of the possibility of graft rejection. GH enhances immune function in rodents [1], and there are GH and insulin-like growth factor 1 (IGF-1) receptors on lymphocytes and macrophages [2]. There are case reports of acute rejection occurring after the introduction of rhGH treatment [3, 4]. We studied lymphocyte subsets and markers of T cell activation before and during rhGH treatment in children with renal transplants, to

see if there were any changes that might predispose to acute rejection.

Patients and methods

Ten patients (7 boys) who were part of a randomised controlled trial of rhGH post renal transplantation [5] were seen on one to four occasions in the year before rhGH treatment (control phase) and 3 monthly during treatment (rhGH phase). Mean (range) age at entry to the study was 12.3 (9.4–15.2) years, glomerular filtration rate (GFR) was 61 (26–93) ml/min per 1.73 m², height standard deviation score –2.7 (–3.5 to –1.6) and height velocity 4.2 (2.6–5.6) cm/year during the preceding year. The mean time from transplantation was 5.2 (1.9–10.2) years. Diagnoses were renal dysplasia in 4 patients, prune-belly syndrome in 2 patients and 1 case each of cystinosis, haemolytic uraemic syndrome, congenital nephrotic syndrome and idiopathic crescentic glomerulonephritis.

Immunosuppression was prednisolone [10.2 (7.6–20.2) mg/m² every other day], azathioprine (60 mg/m² per day) and in seven children cyclosporin A (trough levels 50–150 ng/ml). No child received recombinant erythropoietin. rhGH (Genotropin, Pharmacia and Upjohn, Stockholm, Sweden) 0.14 IU (50 µg)/kg per day was given as a subcutaneous injection each evening. Informed consent and ethical approval were obtained.

At each visit haemoglobin, platelets, differential white count, lymphocyte subsets and T cell activation markers were measured. The number of lymphocytes, T (TCRαβ), CD4⁺, CD8⁺, B and natural killer (NK) cells were calculated. The numbers of T cells expressing the interleukin-2 receptor (CD25), MHC class II antigens (HLA-DR) and another marker of T cell activation, CD26 (dipeptidylpeptidase), were recorded. These three antigens increase during T cell activation at early, intermediate and late times, respectively. Episodes of illness and graft rejection (as diagnosed by the clinician in charge) were recorded.

Lymphocytes were separated on Lymphoprep (Nygaard, Denmark) and incubated with T10B9 (TCRαβ receptor) together with a second antibody (listed below). Staining was performed by double-fluorescence flow cytometry (Facsan flow cytometer, Becton Dickinson) using phycoerythrin-labelled anti-mouse IgM and fluorescein isothiocyanate-labelled anti-mouse IgG as second layers. Using the lymphocyte gate determined by forward angle and side scatter, the percentage total T cells (TCRαβ), T helper (CD4), T cytotoxic (CD8), B (CD19) and NK (CD16⁺CD7⁺) cells was determined. The absolute number of each lymphocyte subset was then calculated from the paired total lymphocyte count. The percentage of T cells expressing CD25, CD26 and HLA-DR antigens was determined.

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Monoclonal antibodies against CD2 (RFT11), CD4 (RFT4), CD7 (RFT2), CD8 (RFT8), CD25 (RFT5), HLA-DR (RFDR2) were raised in the Department of Immunology, Royal Free and University College Medical School. The following antibodies were also used: T10B9 (TCR $\alpha\beta$), a kind gift from Professor Thompson, Kentucky, USA, CD16 (Leu11b, Becton Dickinson) and CD26 (Ta-1, Coulter).

As lymphocyte counts vary with age, and because it was not possible to find an exactly matched control group, the patients acted as their own controls. To allow for day-to-day variation, the average value of each subset or activation marker was calculated during the control and rhGH phases and compared using a paired Student's *t*-test. To exclude a difference due to the effects of time alone, the change in lymphocyte numbers and activation markers during each phase was calculated and compared. Summary data are expressed as the mean (SE). Correlations were performed using multiple regression analysis. A *P* value of <0.05 was taken to represent statistical significance.

Results

Nine children completed the study; one required dialysis after 11 months of rhGH. Height velocity increased to 8.5 (5.3–11.7) cm/year during treatment ($P<0.001$). GFR was unchanged: 61 (26–93) ml/min per 1.73 m² on day 1 and 61 (24–109) ml/min per 1.73 m² after 1 year. There was no increased incidence of infections compared with the year before the trial (data not shown).

During the year of rhGH treatment, two children had three and three had one rejection episodes. No anti-lymphocyte preparations were given. These same five children had 12 such episodes in the previous year. The other five children had no episodes of graft dysfunction before or during treatment.

Lymphocyte subsets (Table 1)

There was no significant difference in the total number of lymphocytes, nor in T cell (TCR $\alpha\beta$), CD4 or CD8 counts, or CD4/CD8 ratio, between the control and rhGH phases. The B cell count was lower during rhGH treatment than during the control phase, but there was no sig-

nificant difference between the change in B cell number during each phase ($P=0.72$), suggesting an effect due to time alone.

T cell activation markers (Table 1)

Surface expression of CD25 was variable, but was lower during the treatment phase ($P=0.013$). However there was no significant difference between the change in percentage of CD25+ T cells in the control phase compared with the rhGH phase ($P=0.31$), suggesting an effect of time alone. There was no significant change in expression of the CD26 antigen nor of DR antigens on T cells either during the control or treatment phases.

Correlations

There was no relationship between lymphocyte numbers or percentage T cell activation markers and rejection episodes, GFR or prednisolone dose, either before or during rhGH treatment.

Rejection episodes

The five children who had rejection episodes were compared with the five who did not. There were no significant differences in lymphocyte subsets or T cell activation markers either before or during rhGH.

Discussion

We have shown that lymphocyte subsets and the surface expression of T cell activation markers were unchanged during rhGH treatment of children with renal transplants. GH is known to interact with the immune system; mice with hypopituitarism have thymic aplasia [1], and GH replacement not only improves growth but also restores immune competence. In man peripheral lymphocytes express GH and IGF-I receptors [2], and in vitro studies indicate a role for GH in lymphopoiesis [2], as well as the development of other haematopoietic cell lines [6]. With rare exceptions [7], children with GH deficiency have normal indices of immune function [8,9]. GH replacement may transiently decrease the percentage B cells and CD4⁺ cells [9], although not to subnormal levels.

Possible consequences of rhGH treatment in transplanted children are an increase in the incidence of infection or rejection. To date there has been no reported increase in infections with rhGH [5, 10,11], but the risk of rejection remains unclear. Three multi-centre studies showed no increased incidence of rejection during rhGH treatment post renal transplantation [5, 10,11], but suggested risk factors were the use of alternate-day rather than daily steroids [10], and the occurrence of rejection episodes prior to rhGH treatment [11]. All of the patients

Table 1 Lymphocyte subsets and T cell activation markers in ten pediatric renal transplant patients before and after 1 year of recombinant human growth hormone (rhGH)

	Control phase	rhGH phase	
Number ($\times 10^9$ /l)			
Total lymphocytes	1.88 (0.60–3.48)	1.82 (0.57–3.65)	$P=0.78$
T cells	1.40 (0.45–2.81)	1.38 (0.33–2.96)	$P=0.92$
CD4 ⁺	0.80 (0.18–1.80)	0.80 (0.20–1.94)	$P=0.99$
CD8 ⁺	0.52 (0.05–1.19)	0.50 (0.07–1.65)	$P=0.82$
Natural killer cells	0.25 (0.01–1.49)	0.11 (0.02–0.27)	$P=0.33$
B cells	0.11 (0.03–0.25)	0.07 (0.01–0.17)	$P=0.015$
CD4/CD8	2.33 (1.04–3.78)	2.41 (0.75–7.13)	$P=0.91$
Percentage T cells expressing			
CD25	14.7 (3.4–38.5)	7.0 (0.38–13.5)	$P=0.013$
HLA-DR	25.3 (5.7–82.0)	16.0 (2.9–63.3)	$P=0.34$
CD26	38.1 (3.7–82.1)	34.5 (8.8–60.5)	$P=0.65$

in our study received alternate-day steroids, but only those children with rejection episodes in the year before rhGH had rejection episodes during treatment.

One small study has shown that the response to donor tissue of rhGH-treated mixed lymphocyte cultures is augmented in patients with chronic rejection. In our study, half of the patients had rejection episodes during rhGH treatment. However, they were indistinguishable in terms of lymphocyte subsets and T cell activation markers, either before or during rhGH treatment.

In conclusion, we have not been able to identify any changes in peripheral T cell numbers or activation that might predispose to rejection in children with renal transplants treated with rhGH.

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LITERATURE ABSTRACT

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In chronic nephropathies prolonged ACE inhibition can induce remission: dynamics of time-dependent changes in GFR. Investigators of the Gruppo Italiano Studi Epidemiologici in Nefrologia (GISEN Group)

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The Ramipril Efficacy in Nephropathy Core and Follow-Up Study found that ≥ 36 months of continued ramipril therapy decreased substantially the risk of end-stage renal failure (ESRF) in patients with chronic nephropathies and a urinary protein excretion rate ≥ 3 g/24 h. This study investigates the time-dependent changes in GFR in these patients and in control subjects who were randomized to conventional therapy during the core period and switched to ramipril during the follow-up study. Analyses included 150 patients (continued ramipril; $n = 74$; switched to ramipril; $n = 76$) who had at least three GFR measurements (including baseline) during the whole observation period and a subgroup of 43 patients (continued ramipril; $n = 26$; switched to ramipril;

$n = 17$) who had at least six GFR measurements, including at least three in the core and at least three in the follow-up study. Ramipril (1.25 to 5 mg/d) and conventional therapy were targeted at achieving a diastolic BP below 90 mm Hg. The main efficacy variables were GFR and ESRF (need for dialysis). Analysis was by intention to treat. Throughout the study, the mean \pm SEM rate of GFR decline (Δ GFR) was significantly lower in patients continued on ramipril compared to those switched to ramipril (0.51 ± 0.09 versus 0.76 ± 0.10 ml/min per 1.73 m² per month; $P < 0.03$). In patients on continued ramipril who had at least six GFR measured-but not in control subjects— Δ GFR progressively improved with time and, in the cohort with the longest follow-up, decreased from (in ml/min per 1.73 m² per month): 0.16 ± 0.12 (at 15 months) to 0.10 ± 0.05 (at 60 months). This rate was about 10-fold slower compared to patients on conventional therapy during the REIN Core study. Analyses of the individual slopes found that at the end of the follow-up, 10 of 26 patients on continued ramipril therapy had a positive Δ GFR and another 10 patients had an improvement of Δ GFR while on ramipril therapy. Δ GFR significantly improved in parallel with a significant reduction in proteinuria. Changes in Δ GFR ($P = 0.0001$) and proteinuria ($P = 0.04$) were significantly different in the two groups. Baseline characteristics and changes in systolic and diastolic BP and 24-h urine urea and sodium excretion were comparable. The present results offer evidence that in chronic nephropathies, the tendency of GFR to decline with time can be effectively halted, even in patients with remarkably severe disease.